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CHARACTERS, DISTRIBUTION, AND FOOD PLANTS OF PHLEPSID LEAFHOPPER VECTORS OF CALIFORNIA ASTER-YELLOWS VIRUS[1]

DWIGHT M. DELONG[2] AND HENRY H. P. SEVERIN[3]

INTRODUCTION

SEVERIN (1934, 1940)[4] has previously reported that 3 species and 1 physiological race of leafhoppers transmit the California aster-yellows virus. The present paper deals with the characters, distribution, habitat, and food plants of six species of phlepsid leafhopper vectors. A companion paper (Severin, 1945) presents evidence that the transmission of the virus by the 6 species is not specific.

The genus *Phlepsius* was established by Fieber (1866) for a small number of European species. Van Duzee (1892) published the first paper on North American forms and recorded 18 species, including 13 new ones. Later his catalogue (Van Duzee, 1917) recorded 63 species and 1 variety of *Phlepsius*, including 7 species occurring in California. Ball (1918), who recorded the Mexican and Central American species, established 4 new subgenera. Osborn and Lathrop (1923), publishing a synopsis of the genus, discussed 89 North American species and 1 variety of both genera, and recorded 11 species occurring in California. After their paper had appeared, DeLong (1923) discussed the food plants and habitats of some of the North American *Phlepsius*. Ball (1927) discussed the synonomy of North American phlepsids and established 1 new subgenus. A later paper (Ball, 1931) described some new species and varieties of *Phlepsius*. DeLong and Caldwell (1937) in their check list of the Cicadellidae of America, north of Mexico, recorded 69 species and 4 varieties in the genus *Phlepsius*, 5 species occurring in California; and 29 species and 1 variety in the genus *Texananus*, 7 species distributed in California.

DeLong (1938*a*) described 4 new species of *Texananus* and 3 new species of *Phlepsius*. In the same year (1938*b*), he described 3 new species of *Texananus*, one of which, *pergradus*, is a vector discussed in this paper.

DeLong also (1938*c*) studied the male genitalia as distinguishing characters of closely related species, or of species regarded as synonymous with the genus

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[4] See "Literature Cited" for complete citation, referred to in the text by author and date of publication.

Phlepsius of North America. The ventral segment of the female was illustrated as an aid to be used in identification if the males were not included in the series. Keys were constructed for the species in 3 subgenera, including 46 species and 1 variety of *Phlepsius,* 9 species and 1 variety of *Pendara,* 6 species of *Paraphlepsius,* 1 species of the new subgenus *Josanus,* and 1 species of *Zioninus,* or a total of 63 species and 2 varieties all belonging to the typical *Phelpsius* group.

DeLong (1939) discussed the members of the *majestus* group of *Texananus* and described 4 new species. In the following year (1940) he discussed the species of these 2 genera occurring in Mexico, illustrating the genitalia of all the Mexican species and describing 9 new species. Later (1943) he described 3 more new species of western *Texananus,* 1 of which, *latipex,* is also a vector of the aster-yellows virus. Then, again, in two separate papers (1944*a* and 1944*b*) DeLong described 13 more new species of the Mexican fauna of *Phlepsius* and *Texananus,* listing all the species of these 2 genera occurring in Mexico and illustrating the male and female genital structures.

Until 1918, all the leafhoppers with brown ramose pigment lines were placed in *Phlepsius;* the whole group commonly was called the phlepsids. That year, Ball separated the narrow-headed forms, which he named *Texananus* Because the European-type species is narrow headed, the name *Texananus* is likely to become a synonym of *Phlepsius,* whereas the broad-headed group will probably become *Paraphlepsius.*

GENERAL DISTRIBUTION

The species of *Phlepsius* and *Texananus* reach their greatest known abundance in the temperate regions of North America. Probably the greatest number of species would be found in the lower or central Mississippi Valley. Examining the genus *Phlepsius* proper, one finds that approximately half of the known North American species occur in the southeastern states, whereas about one fourth are northeastern in distribution. When compared with this eastern predominance of species, about 7 per cent occur in the southwestern states, 7 per cent in the plains states, and 13 per cent in the western United States and western Canada.

If the species of *Texananus* are considered in like manner, the predominance is reversed, for these are most abundant in the western and southwestern states. More than 35 per cent of the group are southwestern in distribution, and slightly less than that number are western and northwestern. Only 5 per cent are southeastern, approximately the same number are plains species, and some 15 per cent are northeastern in distribution. Apparently, then, the genus *Phlepsius,* comprising about twice as many species as *Texananus,* is predominantly eastern; *Texananus* is predominantly western. This supposition is borne out by recent work upon the Mexican fauna of this group: the species of *Texananus* are fairly abundant in Mexico; those of *Phlepsius* are rather scarce. Were it not for the fact that the subgenus *Tropicanus,* together with several recently described species, is definitely tropical, the genus *Texananus* would be decidedly predominate in distribution.

Even if the species of *Tropicanus* are included, the genus *Phlepsius* is still represented in Mexico by less than one fourth the number occurring in the

United States, whereas two thirds as many species of *Texananus* have been recorded for Mexico as for this country.

In connection with the western distribution of these two groups, one notes that 5 of the 6 vectors of the aster-yellows virus in California belong to the genus *Texananus*.

HABITAT AND FOOD PLANTS

The habitat may range from bog or marsh to the arid southwestern plains. Most of the species feed upon grass or herbaceous plants and are found under varying conditions. Some occur on the herbaceous growth of the pine woodland floor, and several are abundant in swamp and marsh. Certain of these species are common in the Florida everglades, either on the maiden cane-sawgrass association or in the moist prairies. In the northern fresh-water marsh, several species occur on the *Carex-Phragmites* association or the *Juncus-Eleocharis-Scleria* association. Still others are found in a prairie-grass habitat. A few species occur in the sphagnum-tamarack bog association, whereas others are manzanita species, confined to *Arctostaphylos* and similar types of vegetation.

Though few species live upon trees, one interesting group is the brown-and-white-banded species, all of which feed upon pine. Certain species live upon the southern pines, *Pinus palustris, P. caribaea,* and the like; others live upon the northern white pine, *P. Strobus;* and still other northern species on *P. rigida* and *P. virginiana.* Certain phlepsid species, distinct from those in the United States, are found harbored by the Mexican species of pine.

The meadow, upland pasture, and other grazing areas support large populations of several species. *Phlepsius irroratus* (Say) is both the most common and the most widely distributed species of the genus. The character of the meadow may vary to a high degree. The grassy area may be high or rolling and well drained, or it may be a low, wet meadow; in consequence, the kind of plant and the humidity factor may vary decidedly. Such conditions determine largely the occurrence and abundance of different species in the various areas of the United States. Thus each of the species of *Poa, Andropogon, Aristida, Bouteloua,* and other grasses has its specific kinds of phlepsids.

The optimum habitat for several species is found in open woodland areas of the deciduous forest, especially in the mixed mesophytic forest, where the humidity and ground moisture are rather high and there is a rich growth of herbaceous plants. Some species live in more restricted parts of these forests, such as the ground layer of a mixed hemlock-beech woodland. The *Texananus majestus* group occurs only in the more densely shaded woodlands, on the herbaceous ground layer.

IDENTIFICATION AND SEPARATION OF SPECIES

The narrow-headed species, being similar in color and external appearance, cannot be easily distinguished by these characters. The last ventral segment of the female abdomen and the chitinous structures of the genital chamber, particularly the aedeagus, are the most dependable means of separating the species of this group. Illustrations of these genital structures are appended as an aid in identifying or separating these species of *Phlepsius* and *Texananus*.

Key to the Six Species

1. Head as wide as pronotum..................................*Phlepsius apertinus*

—Head narrower than pronotum.. 2

2. Smaller in size, not exceeding 5.5 mm.................................. 3

—Larger in size, 6 mm or more in length.................................. 4

3. Female segment with a rather broad, rectangular median notch. Male styles tapered, narrow on apical third, the sickle-shaped portion of aedeagus narrow, with a long, slender, basal "handle" and without a thumb at base of curved portion............ *Texananus lathropi*

—Female segment with a short, narrow V-shaped notch at middle of broadly excavated portion. Male styles broad on apical third; the sickle-shaped portion of aedeagus with a short, broad basal "handle" and a long thumb protrusion at base of curved portion extending more than halfway to apex.........................*Texananus pergradus*

4. Female segment slightly produced at middle, forming a blunt tooth on either side of median V-shaped notch. Male plates long, tapered to acutely pointed apices, which reach or exceed apices of pygofer...........................*Texananus spatulatus*

—Female segment broadly excavated on posterior margin, not produced at middle. Male plates short, rounded at apex or with blunt tips, not reaching apex of pygofer...... 5

5. Female segment broadly excavated, without a median notch. Male plates broadly rounded, together semicircular in shape. Aedeagus ventral process short, thick, with prominent dorsally and ventrally produced processes at blunt apex. The dorsal portion is a small, sickle-shaped process........................*Texananus oregonus*

—Female segment broadly excavated, with a short, median V-shaped notch. Male plates short, divergent at apex, and bluntly pointed. Aedeagus ventral process slender, simple, elongate. The dorsal portion is sickle-shaped, with apex bifid.*Texananus latipex*

PHLEPSIUS APERTINUS OSBORN AND LATHROP

(Plate 1)

The head of *Phlepsius apertinus* is a little wider than the pronotum, and the anterior portion of the elytra appears to have a pale band. The length is 6 to 6.5 mm. The vertex is bluntly angled anteriorly and acutely angled, with the front forming a rather definite margin. The length at the middle is less than half the basal width between the eyes. The vertex, pronotum, and scutellum are white, with brown or black irrorations. There is a pale, irregular band in front of the middle of the clavus. There are a few large, whitish areas on the apical portion of the clavus and elytra. The costa is marked with several black spots. The front is black at the base; it is paler, with broken arcs, below.

In the female the last ventral segment slopes on the lateral margins to the rounded posterior angles. The posterior margin is broadly, deeply excavated almost to the base of the segment, the excavation being broadest at the base. The margins of the apex of the excavation are formed by broad, toothlike, pointed projections extending from the posterior margin at either side. The male plates are elongate, broad at the apex, rounded to the outer margins. The styles are long and narrow, extending almost to the apex of the plates, tapered to bluntly pointed apices. The aedeagus is rather short, broadened at the base, and tapered to a pointed apex. In a lateral view it appears barbed, with one barb close to the apex on the posterior margin and another barb more basal on the anterior margin. There is a short process near the base, extending anteriodorsally. The pygofer bears a long, slender spine on the ventro-caudal margin.

Geographic Range.—Phlepsius apertinus is found in Oregon and California.

Distribution and Food Plants in California.—This species of leafhopper is common in fields of alfalfa (*Medicago sativa*) in the Santa Clara, San Joaquin, and Sacramento valleys. The localities in which the food plants grow and the populations were collected are as follows:

Sonoma County: A small number of adults were swept from pasture vegetation at Triniti, on June 4, 1943, by N. W. Frazier.

Santa Clara County: In Gilroy, on May 10, 1943, a few adults were captured in an alfalfa field by J. H. Freitag.

Sacramento County: In a locality known as the "Sacramento Pocket," on September 5, 1941, many adults were taken by H. H. P. Severin and N. W. Frazier, by sweeping cocklebur (*Xanthium canadense*), which grew in the shade of trees. On September 12, 1941, also, Severin found adults in abundant quantity in fields of alfalfa and Ladino clover (*Trifolium repens* L. var. *latum* McCarthy), and wild licorice (*Glycyrrhiza lepidota*).

Merced County: Two females were swept from pasture vegetation by N. W. Frazier on the summit of Pacheco Pass, May 6, 1943.

Madera County: At Madera, on October 9, 1941, a few adults were caught in an alfalfa field by N. W. Frazier.

Fresno County: On October 9, 1941, at Fresno and Selma, a small number of adults were taken in alfalfa fields by N. W. Frazier.

Tulare County: On May 4, 1941, at Woodlake, many adults were collected in an alfalfa field by N. W. Frazier. Later, on June 1 and on July 4, 1944, adults were commonly taken in a grassy Ladino clover field by J. H. Freitag. At Exeter, on October 9, 1943, adults were collected in abundant numbers by N. W. Frazier on bur-clover (*Medicago hispida*), as well as on annual yellow sweetclover (*Melilotus indica*), on common foxtail (*Hordeum murinum*), and on certain grasses.

TEXANANUS LATHROPI BAKER

(Plate 2)

Texananus lathropi is one of the short, broad species of the *ovatus* group. The length is 5.0 to 5.5 mm. The vertex is short and blunt, almost twice as wide between the eyes at the base as the median length; the margin is thick. The vertex, pronotum, scutellum, and inner border of clavus are yellow, with fuscous irrorations. The elytra is whitish and hyaline, marked with dark brown and fuscous. The costal area has darker spots.

The female segment is short and is broadly, shallowly excavated, with a deep quadrangular excavation at the middle.

The male valve is broadly triangular; the plates are strongly convexly rounded to a produced blunt apex on the inner margin. The styles are broad at the base, concavely rounded on the outer margin, and tapered to blunt apices, which are turned outward. The aedeagus is composed of two parts, the lower part being rather long and narrow in lateral view, tapered to a pointed apex, and the dorsal part being sickle-shaped, with a rather long, straight basal portion.

This species was first described as *Phlepsius annulatus* (Osborn and Lathrop, 1923); but Baker (1925), finding that name preoccupied, named the leafhopper *P. lathropi*.

Geographic Range.—One female was reported to have been collected from the base of Mt. Hood, and one male from Alsea Mountain (Oregon) on undergrowth at the edge of a fir forest. The species was taken at Los Angeles, California, by D. W. Coquillett; and in Nevada by C. F. Baker (Baker collection).

Distribution and Food Plants in California.—The localities and food plants of *Texananus lathropi* in California are as follows:

Sonoma County: At Triniti, on June 25, 1939, a mixed population containing 22 specimens of *Texananus lathropi* and *T. latipex* was collected by N. W. Frazier on drying pasture vegetation in a mountainous area at an elevation of 1,800 feet. Later collections in the same locality were made by Frazier as follows: May 29, 1942, when 10 females were captured on bur-clover, wild pea (*Lathyrus Bolanderi*) and *Thermopsis macrophylla;* on June 4, 1943, when 1 female was taken on wild pea; and on June 8, 1944, when 1 male was caught on pasture grasses and weeds.

Alpine Valley: On March 28, 1942, N. W. Frazier collected 8 females on bur-clover.

Marin County: At San Rafael, April 5, 1943, 1 female was collected, probably on wild pea, by N. W. Frazier. At Montara, July 31, 1942, 1 female was captured on tree lupine (*Lupinus arboreus*), by H. H. P. Severin.

Napa County: N. W. Frazier collected 1 female on wild pea at Dry Creek School on April 5, 1943.

Merced County: N. W. Frazier also caught 1 female on pasture vegetation at the entrance to Pacheco Pass on December 19, 1941.

Tulare County: At Exeter, October 10, 1939, 1 male and 3 females were taken on puncture vine (*Tribulus Terrestris*) by H. H. P. Severin.

This leafhopper occurs in the fog belt and in the interior regions of California. The dates of collection indicate that it overwinters in the adult stage.

TEXANANUS PERGRADUS DeLONG

(Plate 3)

This is a small species of the *ovatus* group. The length is 4.5 to 5.5 mm. The vertex is bluntly angled, about 1½ times as wide between the eyes at the base as the median length. The color is variable. In darker specimens a brown spot occurs on either side of the apex, and a broken brown band between the eyes on the disc. The scutellum has four brown spots on the anterior margin. The elytra is pale, with dark-brown ramose pigment lines. There are three pale, distinct spots along the commissure.

The female last ventral segment is rather strongly, broadly excavated, with a short V-shaped notch at the apex of the excavation.

The male valve is produced and triangular. The plates are short, broad, strongly, convexly rounded, together appearing semicircular. The styles are elongated, narrowed except at the base. The apex is bluntly angled. The aedeagus in lateral view shows a slender, delicate, ventral process and a broader sickle-shaped dorsal process, which has a long prominent thumb at the base of the curved portion.

Geographic Range.—The states in which *Texananus pergradus* was collected include Oregon, California, Utah, Colorado, Arizona, New Mexico, Texas, and Mexico.

Distribution and Food Plants in California.—As determined by N. W. Frazier, the localities and food plants of this leafhopper in California are as follows:

San Luis Obispo County: Two males and 1 female were caught in pasture and abandoned vineyards at Santa Maria on June 12, 1942.

Ventura County: On June 11, 1942, at Cavin Station, 3 miles east of Fillmore, 27 males and 24 females were collected on annual yellow sweetclover and annual burweed (*Franseria acanthicarpa*), growing in the Santa Clara river bottom.

Orange County: On June 5, 1942, at Anaheim, 2 males and 1 female were swept from annual burweed growing in the Santa Ana river bottom.

Riverside County: At Banning, June 5, 1942, 3 males and 12 females were collected on annual burweed.

San Diego County: At Boulevard, 5 males and 5 females were taken on *Franseria* sp. on June 7, 1942.

San Bernardino County: On June 5, 1942, at the entrance to Cajon Pass, 1 male and 2 females were captured on *Lotus* sp.

Tulare County: On pasture vegetation along a riverbank at Woodlake on May 4, 1940, 12 males and 14 females were collected.

San Joaquin County: At Byron, November 12, 1942, 1 male was swept from Bermuda grass.

TEXANANUS SPATULATUS VAN DUZEE

(Plate 4)

This is the largest and most common species of the genus in the southwestern United States and Mexico. The length is 6.5 to 7.0 mm. The vertex is produced and bluntly angled, almost twice as wide between the eyes at the base as the median length. The margin is thick and rounded. The color is brown. The vertex has a mottled transverse band between the eyes, anterior to which are two triangular mottled spots.

The female last ventral segment is short, with broadly rounded lateral angles. The posterior margin is slightly produced at the center and bears a V-shaped notch extending about one fourth the distance to the base. The posterior margin is broadly, shallowly excavated on either side, between the lateral angles and the produced tooth on each side of the median notch.

The male valve is short and triangular. The plates are long, tapered to acutely pointed tips, which extend to the apex of the pygofers. The styles, broad at the base, are rapidly narrowed to slender apical portions, which are pointed on the outer apical margins. The aedeagus has a pair of long, slender ventral processes and a dorsal process that arises at the base, is bent caudad, and forms a ventral and dorsal branch at about half its length. The ventral branch is long, slender, and acutely pointed.

Geographic Range.—The distribution of this species includes California, Colorado, Kansas, Texas, and Mexico.

Distribution and Food Plants in California.—The localities and food plants of this leafhopper in the San Joaquin Valley were as follows:

Merced County: At the summit of Pacheco Pass, May 6, 1942, 136 adults were collected on pasture vegetation by N. W. Frazier.

Tulare County: On October 10, 1941, at Ivanhoe, 4 males and 8 females were collected on rough pigweed (*Amaranthus retroflexus*), by H. H. P. Severin, N. W. Frazier, and J. H. Freitag. At Exeter, on October 10, 1941, 1 male was captured on puncture vine, by H. H. P. Severin; on April 1, 1942, 5 males and 5 females were taken on redstem filaree (*Erodium cicutarium*), by N. W. Frazier; and at Woodlake, on January 8, 1942, he collected 3 males, 3 females, and 28 nymphs from redstem filaree, and from whitestem filaree, (*E. moschatum*).

Kern County: At Delano, October 10, 1941, 2 males and 1 female were caught in an alfalfa field by H. H. P. Severin. On December 21, 1941, at Arvin, 62 males and 68 females were taken below leaves of nettle-leaf goosefoot (*Chenopodium murale*), and also on grass under leaves in grape vineyards, by N. W. Frazier and J. H. Freitag.

Evidently this leafhopper overwinters in the nymphal and adult stages and undergoes an incomplete hibernation.

TEXANANUS OREGONUS BALL

(Plate 5)

Texananus oregonus is a rather robust species, 6.5 mm. in length. The vertex, produced and bluntly angled, is about two thirds as wide between the eyes as the median length. It is pale brown, with black markings at the ends of the claval veins along the posterior margin of the elytra. Although it resembles *superbus* and other species of the genus, the genital structures will easily distinguish it from allied species.

The female last ventral segment is short and broad. The lateral margins slope to rounded posterior angles. The posterior margin is broadly, shallowly excavated, and brown-margined.

The male valve is short and triangular, with a blunt apex. The plates are short, broadly rounded from base to apex. Together they appear semicircular. The pygofers are a little longer than the plates. The styles are broad on the basal two thirds; the apical third is abruptly narrowed and produced as a narrow portion on its inner margin. The aedeagus is bifid at the apex, with a dorsal process arising near the base, which is sickle-shaped on the apical portion. The aedeagus proper is rather short, in lateral view broadened at the apex, with a dorsally directed and a ventrally directed pointed process. The apex is truncate.

Geographic Range.—This leafhopper has been recorded from Washington, Oregon, California, and Mexico.

Distribution and Food Plants in California.—As determined by N. W. Frazier, the localities and food plants of populations collected in California, are as follows:

Sonoma County: At Triniti, on May 12, 1940, 10 males and 12 females were taken on bur-clover and wild pea. On March 28, 1942, 1 male and 5 females were taken on wild pea. On February 13, 1943, 2 males and 1 nymph (a female after the last molt) were captured on an annual species of *Lotus* and on wild honeysuckle (*Lonicera hispidula* Dougl. var. *californica* Jepson). On April 7, 1943, 1 female was swept from wild pea. On June 8, 1944, 2 nymphs (1 male and 1 female after the last molt) were caught on pasture grasses and weeds. At Alpine Valley, on March 28, 1942, 4 females were collected on bur-clover.

Napa County: Two females were taken on wild pea at Dry Creek School, February 13, 1943.

The collection data show that the adults winter over.

TEXANANUS LATIPEX DeLONG

(Plate 6)

This is one of the short, broad phlepsids of the *ovatus* group. The length is 6 mm. The vertex is bluntly angled, almost twice as wide between the eyes at the base as the median length. The vertex, pronotum, and scutellum are yellowish, marked with brown ramose pigment. The basal angles of the scutellum have dark-brown spots. The elytra are more heavily marked with brown pigment, with three distinct commissural spots.

The female last ventral segment is short, with rather prominent lateral angles, between which the posterior margin is rather deeply, broadly excavated, with a slight median V-shaped notch.

The male valve is triangular. The plates are short, convexly rounded to bluntly pointed, produced, diverging apices. The styles are elongated, concavely curved, and tapered to narrow blunt apices. The aedeagus is composed of an upper and a lower portion. In lateral view the ventral portion is rather long, narrow, and tapered to an acute tip. The dorsal portion is broad, strongly curved, sickle-shaped, broadened apically, and bifid at the apex, forming two stout teeth.

Geographic Range.—Texananus latipex occurs in British Columbia, Washington, Montana, North Dakota, Oregon, Idaho, Wyoming, California, Nevada, Utah, Colorado, Arizona, New Mexico, Texas, and Mexico.

Distribution and Food Plants in California.—The localities in which this leafhopper was collected in California and its food plants are as follows:

Sonoma County: At Triniti, on June 25, 1939, a mixed population of *Texananus latipex* and *T. lathropi* was collected on drying pasture vegetation in a mountainous area at an elevation of 1,800 feet. One female was taken on pasture vegetation July 14, 1943, in the same locality. At Alpine Valley, March 28, 1942, 1 female was captured on bur-clover by N. W. Frazier.

San Mateo County: On September 19, 1941, at Half Moon Bay, 1 female and 1 nymph (a female, after the last molt) were taken on spiny clotbur (*Xanthium spinosum*) by H. H. P. Severin.

Santa Clara County: At Milpitas, February 19, 1943, 3 females were caught in a depleted, grassy alfalfa field by J. H. Freitag. He also collected 1 female in an alfalfa field at San Jose on March 4, 1942; and 1 female on grasses in a bog at Gilroy on August 11, 1942.

Alameda County: At Pleasanton, June 30, 1943, 1 female was captured in an alfalfa field by J. H. Freitag.

Madera County: On October 9, 1941, at Madera, 1 female was taken in an alfalfa field by H. H. P. Severin. At Chowchilla, December 19, 1941, 4 females and 2 males were collected on pasture vegetation by N. W. Frazier.

Fresno County: At Fresno, November 17, 1941, 4 females and 2 males were caught on redstem filaree, and 2 females were collected on Bermuda grass (*Cynodon Dactylon*), on April 22, 1942. At Raisin City, on December 22, 1941, 1 female was taken on grass by N. W. Frazier.

Tulare County: At Exeter, on October 9, 1941, H. H. P. Severin captured 1 female in an alfalfa field and 2 females on puncture vine. At Visalia, June 18, 1942, 1 female was caught on inland saltgrass (*Distichlis stricta*), and at Ivanhoe-Elderwood, April 29, 1943 was collected 1 female on pasture vegetation by N. W. Frazier.

Kern County: On October 10, 1941, at Delano, 1 female was collected in an alfalfa field by H. H. P. Severin.

Evidently *Texananus latipex* occurs in the fog belt, in the San Joaquin Valley, and in the mountains of Sonoma County. This insect overwinters in the adult stage.

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PLATES

PLATES

1. *Phlepsius apertinus* Osborn and Lathrop
2. *Texananus lathropi* Baker
3. *Texananus pergradus* DeLong
4. *Texananus spatulatus* Van Duzee
5. *Texananus oregonus* Ball
6. *Texananus latipex* DeLong

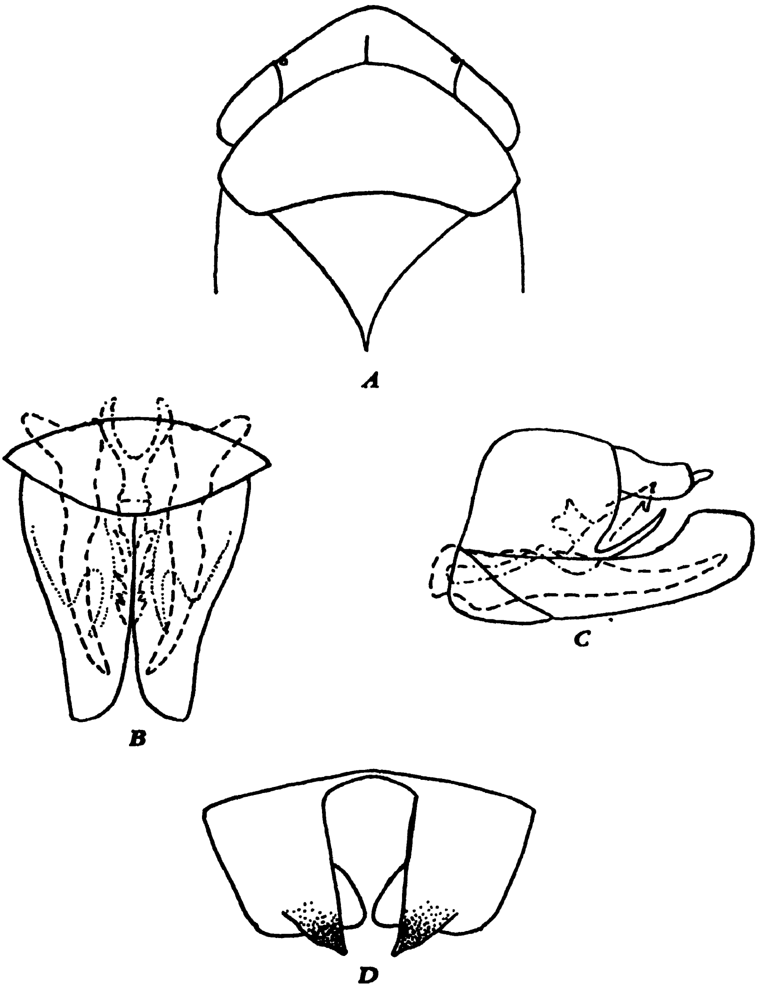
KEY TO FIGURES ON PLATES

A—Dorsal view of head, pronotum, and scutellum
B—Ventral view of male genitalia
C—Lateral view of male genitalia
D—Ventral view of female last ventral segment

KEY TO FIGURE DETAIL OUTLINES

- - - - - - -	style
. — . — . — .	aedeagus
....	dorsal spine of pygofer

PHLEPSIUS APERTINUS



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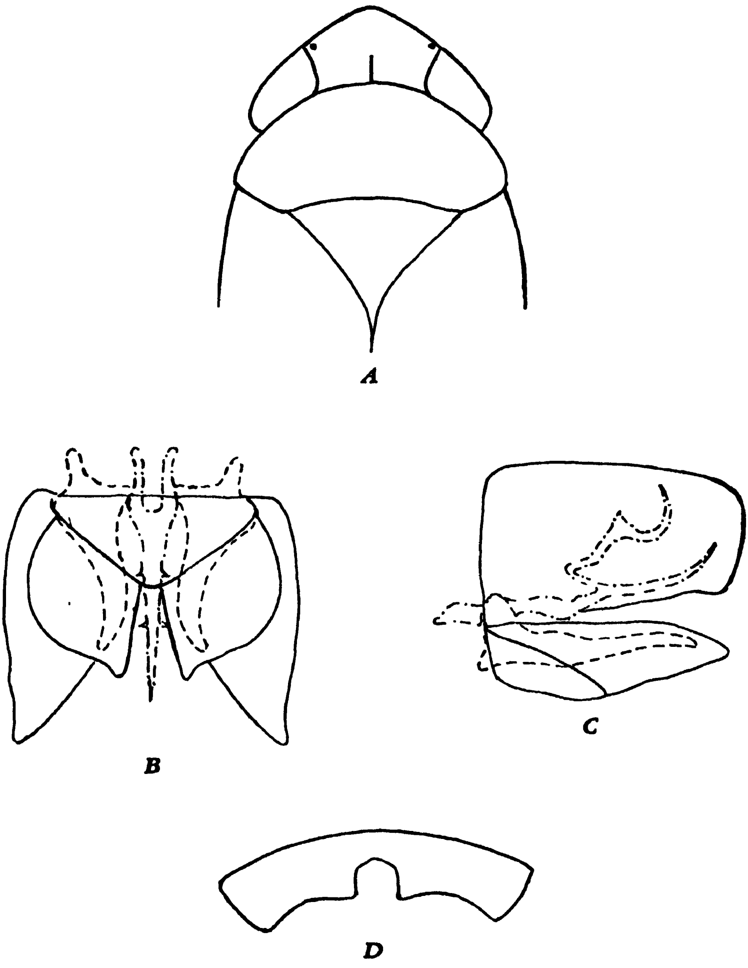
TEXANANUS LATHROPI



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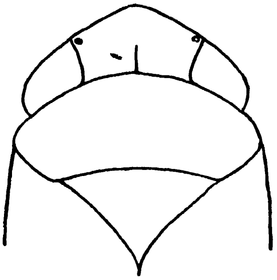
TEXANANUS PERGRADUS



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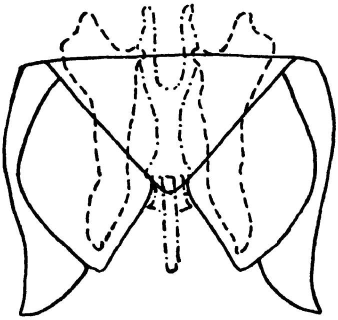

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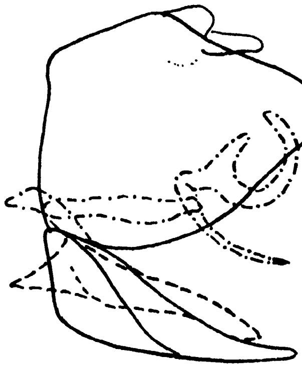

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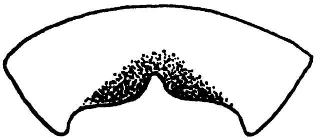

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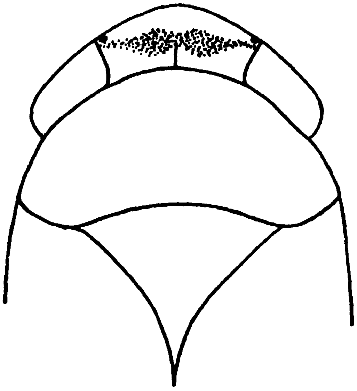
TEXANANUS SPATULATUS



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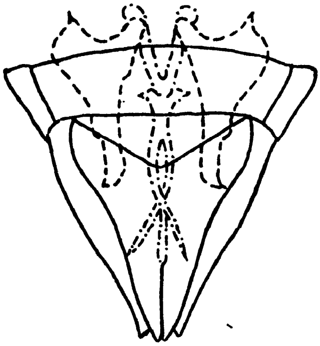

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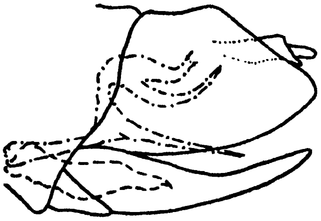

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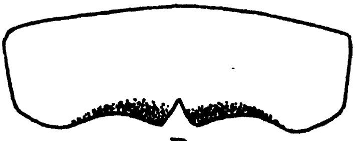

D

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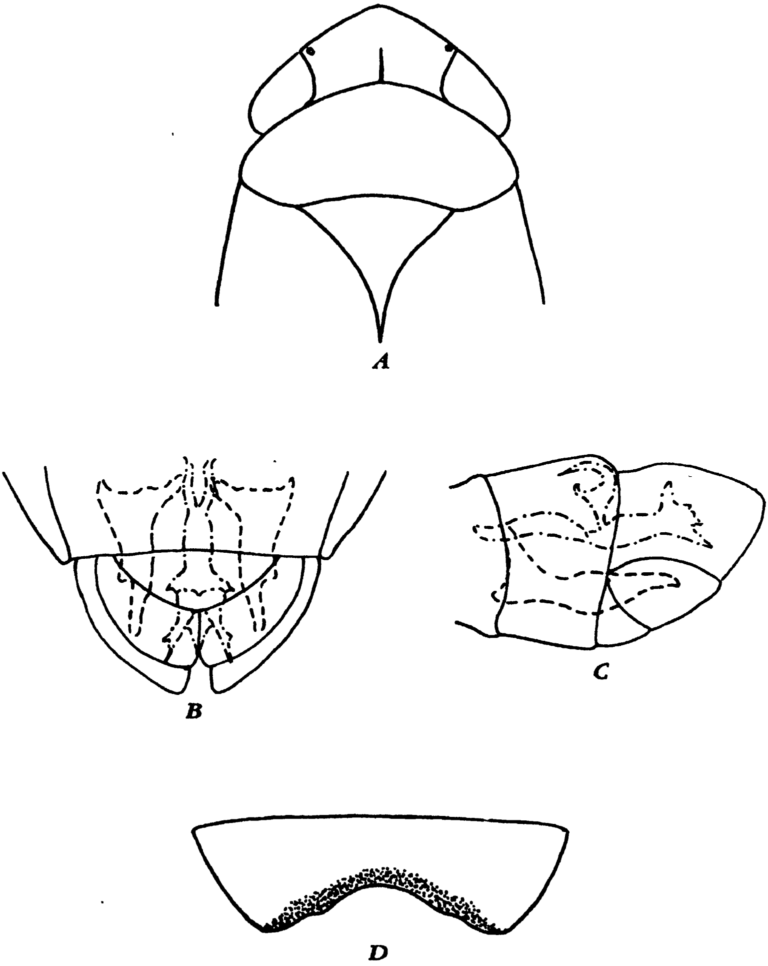
TEXANANUS OREGONUS



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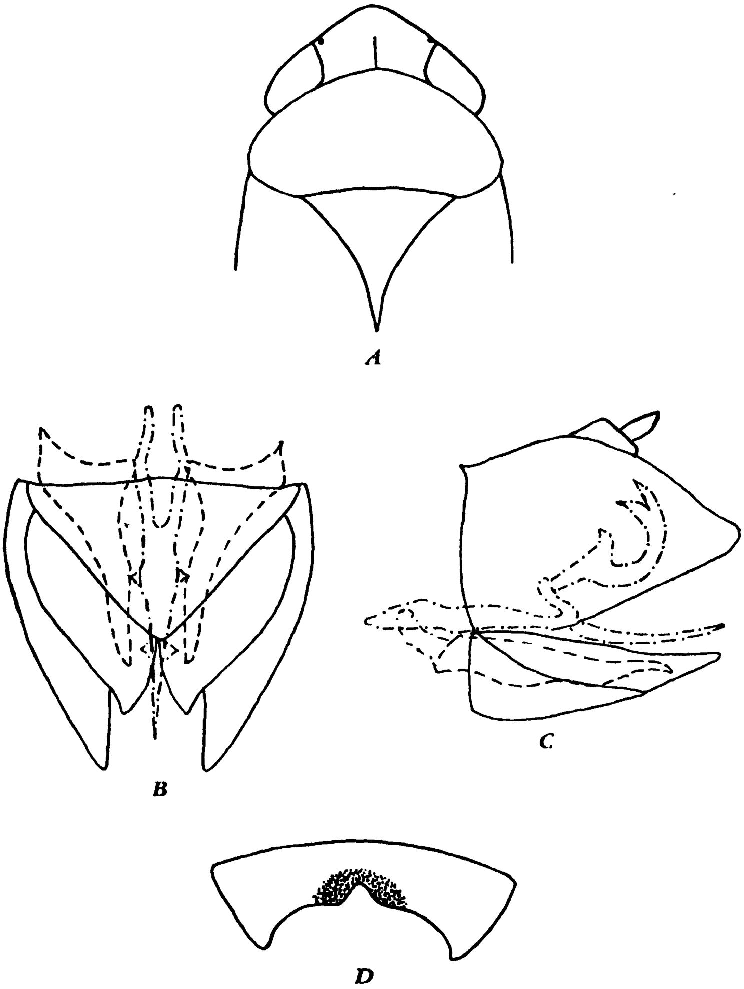
TEXANANUS LATIPEX



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EVIDENCE OF NONSPECIFIC TRANSMISSION OF CALIFORNIA ASTER-YELLOWS VIRUS BY LEAFHOPPERS

HENRY H. P. SEVERIN

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EVIDENCE OF NONSPECIFIC TRANSMISSION OF CALIFORNIA ASTER-YELLOWS VIRUS BY LEAFHOPPERS[1]

HENRY H. P. SEVERIN[2]

INTRODUCTION

IT MAY BE NECESSARY to define two terms before proceeding with this paper. Specificity is the transmission of a particular virus by one or two closely related species of vectors. "When a virus can be transmitted only by one or two closely related insects and cannot be transmitted artificially (except by grafting) the insect transmission is considered obligatory" (Leach, 1940).[3]

Formerly it was assumed that a specific relation existed between each plant virus and its aphid vector. Among the viruses transmitted by aphids, specificity is now regarded as exceptional rather than common.

In the opinion of Smith (1937), a rather strong point in favor of a specific relationship between leafhopper and virus lies in the fact that certain plant viruses can be transmitted by only one species. Heretofore it has been assumed that the aster-yellows virus could be disseminated only by the aster leafhopper, *Macrosteles divisus* (Uhl.) = [*Cicadula sexnotota* (Foll.)], and by no other means except grafting and budding of the plant.

According to Kunkel (1926), the intimate and specific relation existing between the aster-yellows virus and its insect vector is important evidence that the causative entity is biological rather than chemical. Leach (1940) emphasizes the biological, obligatory, and highly specific transmission of viruses by leafhoppers.

There may be some viruses which can be transmitted by only one species of leafhopper. During the past thirty years many species of leafhoppers collected in sugar-beet fields were tested by the author for the transmission of the curly-top virus, but up to the present time no other vector is known except the beet leafhopper, *Eutettix tenellus* (Baker). According to DeLong (1942), the North American fauna of leafhoppers, north of Mexico, consists of 2,000 species belonging to about 150 genera. No other species of the genus *Eutettix* has been tested up to the present time. It is naturally assumed that closely related species in the same genus should be tested; on the other hand, species in genera belonging to different subfamilies may be vectors. The sugar beet is an introduced plant, and it may be possible that if leafhoppers are collected on native host plants of the virus, other vectors may be discovered. As will be discussed with aster yellows in this paper, host plants highly susceptible to the virus should be used instead of the sugar beet.

Herewith is presented evidence that the transmission of the aster-yellows virus by six species of phlepsid leafhoppers is not specific. The characters, distribution, and food plants of these six vectors are discussed in a companion paper (DeLong and Severin, 1945). It is evident in the following review of literature that the prevailing concept has in general favored specificity.

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[1] Received for publication November 8, 1944.

[2] Entomologist in the Experiment Station.

[3] See "Literature Cited" for complete citation, referred to in the text by author and date of publication.

REVIEW OF LITERATURE ON LEAFHOPPER-TRANSMITTED VIRUSES

Aster Yellows.—Severin (1929) reported the transmission of the California aster-yellows virus to celery, lettuce, and ornamental flowering plants by the short-winged aster leafhopper. Severin and Haasis (1934) showed that potato could be experimentally infected with the virus by means of the short-winged aster leafhopper. The virus was not recovered by previously noninfective leafhoppers from infected potato plants, nor from potato tubers obtained from plants showing symptoms of aster yellows. Severin (1940) proved that potato was naturally infected; also that the virus was recovered and transferred to healthy asters by the long-winged aster leafhopper, a race of the same species. Severin (1934) reported the transmission of the California aster-yellows virus by the mountain leafhopper, *Colladonus montanus* (V.D.) = (*Thamnotettix montanus* V. D.), and by the geminate leafhopper, *Idiodonus geminatus* (V. D.)=(*T. geminatus* V. D.). In a recent paper, Severin (1942) stated that the mountain and geminate leafhoppers are the most important vectors of the virus to delphinium, and that both species breed on this plant under natural conditions. Delphinium is an unfavorable food plant of the short-winged and long-winged aster leafhoppers; some of the insects feeding on the plant died within 4 to 6 hours, and most of them within 24.

Sugar-Beet Curly Top.—Ball (1906) associated the abundance of the beet leafhopper with curly top in the beet fields. Considerable controversy appears in the literature as to whether he was the first to prove that curly top was induced by this insect. He caged 14 beets in the field, introduced beet leafhoppers into 7 cages, and used 7 as controls (Ball, 1909). Cages 1 to 3 were failures; the insects escaped. Ball stated "Temperature and moisture could not be controlled and no curly top was produced in the remaining cages." In another experiment, 12 leafhoppers and their eggs stopped the growth of a beet in less than 2 weeks; and they together with their progeny killed it in less than 2 weeks more. A footnote reports the occurrence of curly top in cages arranged by T. G. Titus in joint investigations with Ball. Shaw (1910) demonstrated that the beet leafhopper caused curly top of sugar beets in cages in the field. The leafhopper occurs in British Columbia (Davis, 1927), western United States (Severin, 1933), Lower California (Severin and Henderson, 1928), and western Mexico as far south as Guasave, Sinaloa (California Agricultural Experiment Station, 1928).

Fawcett (1927) reported a similar curly-top disease of sugar beets in Argentina—a different strain of the virus, transmitted by the leafhopper *Agalliana ensigera* Oman. This leafhopper was first determined as *Agallia sanguinolenta* (Prov.) by McAtee in 1928 and later (during the same year) as *A. sticticollis* Stal by Osborn and Ball. *A. sticticollis* is the species referred to by Fawcett (1925, 1927) and by Severin and Henderson (1928); but Oman (1934), examining numerous specimens from Fawcett and Henderson, failed to find Stal's species in any of the material. There seems to be at present no reason to believe that *A. sticticollis* occurs in the sugar-beet-growing regions of the Argentine Republic.

Pierce's Disease of Grapevine and Alfalfa Dwarf.—Hewitt, Frazier, and Houston (1942) reported that two undetermined species of leafhoppers in the genera *Draeculacephala* and *Carneocephala* transmitted the virus of Pierce's disease of grapevine. In a later paper Hewitt, Frazier, Jacobs, and Freitag (1942) named, as vectors of the virus, the green sharpshooter, *D. minerva* Ball; the redheaded sharpshooter, *C. fulgida* Nott.; and the blue-green sharpshooter, *Neokolla circellata* (Baker) = [*Cicadella circellata* (Baker)]. Frazier (1945) lists the following 6 additional species of leafhoppers as vectors: *Carneocephala triguttata* Nott., *Helochara delta* Oman, *Neokolla gothica* (Sign.), *N. confluens* (Uhler), *N. heiroglyphica* (Sat.), and *Cuerna occidentalis* Oman & Beamer. All are species of the subfamily Amblycephalinae=(Cicadellinae), according to Medler (1942).

Alfalfa Witches'-Broom.—Menzies (1944) has presented evidence that the leafhopper *Platymoides acutus* Say is the vector of the virus of alfalfa witches'-broom. This disease occurs in Washington.

False Blossom of Cranberry.—Dobroscky (1929, 1931) demonstrated that the virus of false blossom of cranberries is transmitted by the blunt-nosed leafhopper, *Euscelis striatulus* Fall. The disease occurs wherever cranberries are grown in the United States.

Peach Yellows.—Kunkel (1933) proved that the vector of peach-yellows virus is the plum leafhopper, *Macropsis trimaculata* (Fitch). The dissemination of the virus in nature had defied the efforts of plant pathologists for forty-five years. Peach yellows is limited in its distribution to the eastern United States.

Potato Yellow Dwarf.—Black (1937) has proved that virus of potato yellow dwarf is transmitted by the clover leafhopper, *Acertatagallia sanguinolenta* (Prov.); and his findings were confirmed by Walker and Larson (1938). In a later paper Black (1941) reported that *A. sanguinolenta* carried a strain of virus from New York and that *Agallia constricta* V. D. transmitted a strain of virus from New Jersey. According to him, "the results indicate that there exists a high degree of specificity, perhaps an absolute specificity, in the relationship between the two varieties of virus and the two related leafhoppers."

Clover Club-Leaf.—In a recent paper, Black (1944) reported that *Agalliopsis novella* (Say) collected near Washington, D. C., transmitted two viruses to crimson clover; but in nature the diseases are unknown.

Dwarf or Stunt Disease of Rice.—The relation of leafhoppers to the dwarf disease of rice was first experimentally proved by Hatsuzo Hashimota, a local grower of Shiga prefecture, in 1884, according to Ishikawa (1928) on the authority of Katsura (1936). Takata (1895–96) concluded that among several leafhoppers investigated, *Deltocephalus dorsalis* Motsch. was not the vector of the virus causing dwarf disease of rice; but in his further investigations in 1900 Takata reported that only the rice leafhopper, *Nephotettix apicalis* Motsch. var. *cincticeps* Uhl., was capable of transmitting the virus. According to Fukushi (1937), Japanese plant pathologists have generally regarded the rice leafhopper as the sole agent that transmits the virus. In his studies on the relation of several species of leafhoppers to the transmission of the virus, *D. dorsalis* proved to be another vector. Dwarf of rice has been a serious disease in Japan for many years.

Agati, Sison, and Abolis (1941) demonstrated that the rice leafhopper, *Nephotettix bipunctatus cincticeps,* is also the vector of the virus of dwarf disease of rice in central Luzon.

Streak of Corn (*Maize*).—Storey (1925*a*, 1928) reported the transmission of the virus of corn by the maize leafhopper, *Cicadulina mbila* (China) = (*Balclutha mbila* Naude). Later he found that two other closely related species, *C. zeal* China and *C. storeyi* China, carry the virus (Storey, 1937). The most efficient vector is *C. mbila.* Evidently, 3 species of leafhoppers belonging to one genus transmit the virus. Streak of corn is a destructive disease in South Africa.

Mottle of Corn (*Maize*).—Storey (1937) discussed the transmission of the virus of mottle of corn by 3 species of leafhoppers—*Cicadulina mbila* Naude, *C. zeal* China, and *C. storeyi* China. He suggests that the mottle and streak viruses may be regarded as two strains of one virus. In his text and summary, however, he states: "The mottle virus is regarded as a new one, unrelated to that of streak disease." This is the first example of 2 viruses being transmitted by the same 3 species of leafhoppers. An insect already carrying one virus may also take up and transmit the other. The disease has been found in Tanganyika Territory.

Streak Disease of Sugar Cane.—Storey (1925*b*) reported that the virus of streak of sugar cane is spread only by the maize leafhopper, *Cicadulina mbila.* By feeding the leafhoppers on the tip of a single leaf of diseased sugar cane and then transferring them to the tip of a single leaf of healthy cane, he caused 5 to 19 plants to develop streak. The virus from corn caused only a transitory infection of sugar cane, from which the plant subsequently recovered. The virus from sugar cane produced in corn a permanent but mild form of streak disease. Storey and McClean (1930) concluded that the virus of corn streak and the virus of cane streak are not identical. Evidently, *C. mbila* transmits 3 viruses.

According to Storey (1926), attenuation of the virus of cane streak occurred in passage from sugar cane to corn by use of the maize leafhopper, *Cicadulina mbila.* The transfer of the virus to corn streak to cane caused only a few chlorotic stripes, and the foliage was healthy thereafter. The cane plants were therefore thought to have been infected by the virus from maize, but to have recovered from it.

Sugar-Cane Mosaic.—No leafhopper is known to transmit a mosaic virus up to the present time. Kunkel (1944) in a review of Bawden's (1943) book called attention to the fact that the virus of sugar-cane mosaic is disseminated by aphids, but is not known to be transmitted by any species of leafhopper. Brandes (1920), Ingram and Summers (1936), Ingram (1937), Ingram, Haley, and Charpentier (1939) reported that *Draeculacephala portola* Ball= [*D. mollipes* (Say) Abbott and Ingram (1942)], failed to transmit the virus of sugar-cane mosaic. The following species of aphids are vectors of the virus: corn aphid, *Aphis maidis* Fitch, reported by Brandes (1920), Kunkel (1922), and Chardon and Veve (1922); rusty plum aphid, *Hysteroneura setariae* (Thomas), reported by Ingram and Summers (1936), Ingram (1937), and Ingram, Haley, and Charpentier (1939); spring grain aphid, *Toxoptera graminum* Rond., reported by Ingram (1937), Ingram and Summers (1938), and Ingram, Haley, and Charpentier (1939); and *Carolinaia cyperi* Ainslie, Tate, and Vanderberg (1939).

Chlorotic Streak of Sugar Cane.—Abbott and Ingram (1942) offer proof that the leafhopper *Draeculacephala portola* is a vector of the virus of chlorotic streak of sugar cane. The disease occurs in the Gulf states.

Yellow Dwarf of Tobacco.—According to Hill (1941), the leafhopper *Thamnotettix argentea* (Evans) is the vector of the virus of tobacco yellow dwarf in Australia.

Wallaby Ear Disease of Corn (Maize).—Schindler (1942) concluded that the leafhopper *Cicadula bimaculata* Evans is responsible for the wallaby ear disease, which is probably caused by a virus. He reported transmission to corn in 2 cages containing *C. bimaculata;* and in 4 of 5 cages containing *C. bimaculata* and the corn leafhopper (fulgorid) *Peregrinus maidis* Ash. In 1 cage containing *P. maidis,* the disease failed to develop.

Big Bud of Tomato.—Hill (1937, 1943) reported that the virus of big bud of tomato and tobacco yellow dwarf was transmitted by the leafhopper *Thamnotettix argentea* (Evans). The list of additional economic plants experimentally infected by *T. argentea* includes eggplant, pepper, sugar beet, white and red clover, also ornamental flowering plants and weeds, a total of 23 species of plants of 13 families. Evidently this leafhopper transmits two viruses. Big bud of tomato occurs in all the Australian states.

Ryjkoff (1935) and Ryjkoff and Karatchevsky (1935) consider tomato woodiness, virescence of tobacco flowers, belladonna, and weeds in the Crimea and Ukraine to be identical with big bud of tomato. They suggest that the leafhopper *Agallia sinuata* is responsible for its spread in the field.

Little-leaf of Eggplant.—Thomas and Krishnaswami (1939) reported that 2 species of leafhoppers disseminate a virus to eggplants. The virus was transmitted from diseased eggplants to 19 of 59 healthy plants by *Eutettix phycitis* Dis.; to 1 of 9 healthy plants by *Empoasca devastens* Dis. The disease causes considerable damage to the crop in south India, resulting in dwarfed leaves and sterility. Hill (1943) considers little-leaf of eggplant to be either the same as big bud of tomato or a closely related disease.

VIRUS TRANSMISSION EXPERIMENTS AND BIOLOGY OF *TEXANANUS LATHROPI* BAKER AND *T. LATIPEX* DELONG

A species of *Texananus* was collected on July 31, 1934, in miscellaneous sweepings on weeds, grasses, and ornamental flowering plants in South Canyon Nursery, near Montara, San Mateo County. A population was reared on celery infected with the California aster-yellows virus, but during the winter the leafhoppers died. This species proved to be *T. latipex* DeLong, described by DeLong (1943). Five years later 22 adults were collected by N. W. Frazier, on drying pasture vegetation in a mountainous area at an elevation of 1,800 feet near Triniti, Sonoma County, on April 25, 1939. A large population was reared on diseased celery. Adults were sent to P. M. Oman, Division of Insect Identification, Bureau of Plant Quarantine, Washington, D. C. Oman[4] found a mixed population containing *T. lathropi* Baker and a new species. Before he could publish a description of the latter, he joined the United States Army. He sent the leafhoppers to R. H. Beamer, University of Kansas, Lawrence, Kansas, for determination. Beamer forwarded the specimens to DeLong, who described the new species as *T. latipex.* Etherized females of the two species were separated from a mixed population by means of a binocular microscope, and the investigations were resumed.

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[4] P. M. Oman in a letter to the author, dated May 6, 1941.

A study was undertaken with the two species of leafhoppers to determine the efficiency of the vectors in transmitting the virus by single males and females, each kept on a healthy celery plant until symptoms of the disease developed, or for 2 months if no symptoms appeared. Varying numbers of adults were kept for different periods on healthy celery and asters and upon successive asters. Attempts were made to determine the latent period of the virus and the retention of the virus in the adults. The ability of the two leafhopper species to transmit the viruses of curly top and Pierce's disease of grapevine was also investigated. Life histories of the two phlepsid species were studied.

METHODS AND EQUIPMENT USED WITH THE TWO VECTORS

Etherized adult males and females of both species were segregated under a binocular microscope. The cages used and the method of transferring leafhoppers in a dark chamber have already been described (Severin, 1930, 1931). Repeated capturing of the leafhoppers results in a mortality and was therefore avoided in all experiments. During transfers of the adults to successive healthy celery plants, the clay pot on which the cage rested was jarred on the table in a dark chamber, and a breath of air was blown between the petioles to free the plant from leafhoppers.

TRANSMISSION OF CALIFORNIA ASTER-YELLOWS VIRUS

To Celery by Single Males and Females.—An experiment was conducted on the transmission of the virus by 150 males and 150 females of *Texananus lathropi,* each kept singly on a healthy celery plant. The males and females had completed the nymphal stages on diseased celery, requiring an average of 59.5 and 66.4 days, respectively, during January and February (table 10). Whenever an infected celery plant became unfavorable as food, the nymphs were transferred to a second diseased celery plant. A total of 300 adults were kept singly on healthy celery. The males infected 28 of 150 plants inoculated or 18.7 per cent and the females 24 of 150 plants or 16.0 per cent.

A similar test was made with 100 males and 100 females of *Texananus latipex,* each kept singly on a healthy celery plant. The males and females completed the nymphal stages on diseased celery, requiring an average of 52.6 days and 56.3 days, respectively, during February and March (table 10). The males infected 18 of 100 plants inoculated and the females 32 of 100 plants.

To Celery Alternating with Asters in Daily and Weekly Inoculations.—The transmission of the virus to successive celery and asters by each species of leafhopper was compared. Lots of 40 recently molted males or females, which had completed the nymphal stages on diseased celery, were transferred daily for 42 to 46 days to healthy celery alternating with asters. In a similar experiment weekly inoculations were made alternately on celery and asters. Table 1 indicates the results.

As this table shows, when the males and females of *Texananus lathropi* were transferred daily to celery alternating with asters, the males infected 20.2 and the females 11.4 per cent of the celery, respectively. When transferred weekly to celery alternating with asters, the males infected 50.0 and the females 45.8 per cent of the celery. Under the same conditions, transmission by *T. latipex* was as follows: daily transfers to celery, males 25.0 per cent and females 22.5; weekly transfers to celery, males 44.4 per cent and females 58.3. Evidently the period of exposure of healthy celery to the leafhoppers is important in the virus transmission.

TABLE 1

TRANSMISSION OF VIRUS TO CELERY ALTERNATING WITH ASTERS IN DAILY AND WEEKLY INOCULATIONS, BY ADULT *Texananus lathropi* AND *T. latipex*

Number of lots	Species and number of adults in each lot	Celery			Asters			Adults alive at end of 6 weeks
		Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected	
	Daily inoculations							
8	*T. lathropi*, 40 males.....	168	34	20.2	168	2	1.2	20-35
8	*T. lathropi*, 40 females....	184	21	11.4	179	2	1.1	21-33
5	*T. latipex*, 40 males.......	116	29	25.0	113	4	3.5	18-33
6	*T. latipex*, 40 females.....	138	31	22.5	135	14	10.4	16-35
	Weekly inoculations							
8	*T. lathropi*, 40 males.....	24	12	50.0	24	0	0.0	18-38
8	*T. lathropi*, 40 females....	24	11	45.8	24	0	0.0	26-37
6	*T. latipex*, 40 males.....	18	8	44.4	18	2	22.2	35-39
4	*T. latipex*, 40 females.....	12	7	58.3	12	0	0.0	18-21

A total of 347 asters were inoculated by lots of 40 males or females of *Texananus lathropi* in daily transfers, and 4, or 1.2 per cent, were infected; but of 48 asters inoculated in weekly transfers, none showed any effect. In daily transfers, lots of 40 males or females of *T. latipex* inoculated 248 asters, infected 18 or 7.3 per cent in daily transfers. They infected 2 of 30 asters, or 6.7 per cent in weekly transfers.

To Asters Alternating with Celery in Daily and Weekly Inoculations.—The retention of the virus by single males and females of the two species of leafhoppers, to be discussed later, indicates that many leafhoppers cause only 1 infection apiece during adult life. For this reason, lots of 40 males and 40 females were transferred daily and weekly during a period of 6 weeks to asters alternating with celery, instead of celery alternating with asters as in the previous experiment. The results appear in table 2: lower percentages of infection were obtained in asters and celery in daily than in weekly transfers with both species of leafhoppers.

To Successive Celery Alternating with Asters, by Varying Numbers of Adults Reared on Infected Celery.—A study was made of the transmission of the virus by single males and by lots of 5, 10, and 20 males of *Texananus latipex* reared on diseased celery and transferred to celery alternating with asters.

TABLE 2

TRANSMISSION OF VIRUS TO ASTERS ALTERNATING WITH CELERY IN DAILY AND WEEKLY INOCULATIONS, BY ADULT *Texananus lathropi* AND *T. latipex*

Number of lots	Species and number of adults in each lot	Asters			Celery			Adults alive at end of 6 weeks
		Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected	
				Daily inoculations				
4	*T. lathropi*, 40 males......	84	9	10.7	84	28	33.3	20–23
5	*T. lathropi*, 40 females....	105	5	4.8	105	29	27.6	21–30
12	*T. latipex*, 40 males.......	252	16	6.3	252	69	27.4	21–32
				Weekly inoculations				
8	*T. lathropi*, 40 males. ..	24	3	12.5	24	14	58.3	25–40
9	*T. lathropi*, 40 females....	24	2	8.3	24	7	29.2	25–36
11	*T. latipex*, 40 males......	27	4	14.8	27	16	59.3	20–36
8	*T. latipex*, 40 females.....	24	8	33.3	24	12	50.0	24–35

TABLE 3

TRANSMISSION OF VIRUS TO SUCCESSIVE CELERY ALTERNATING WITH ASTERS, BY VARYING NUMBERS OF ADULT *Texananus latipex* REARED ON INFECTED CELERY

Number of lots	Number of males in each lot	Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected
		First set of celery			First set of asters		
100	1	100	11	11.0	100	1	1.0
50	5	50	12	24.0	50	2	4.0
50	10	50	37	74.0	50	18	36.0
50	20	50	40	80.0	50	12	12.0
		Second set of celery			Second set of asters		
100	1	..	..	....	..	..	....
50	5	50	4	8.0	50	0	0.0
50	10	50	17	34.0	50	5	10.0
50	20	50	11	22.0	50	4	8.0

Each of the 100 males was kept on a healthy celery plant for 4 weeks and then was transferred to a healthy aster and kept there during adult life. Lots of 5, 10, and 20 males were changed weekly during a period of 4 weeks to successive celery plants alternating with asters. Table 3 shows the results.

One hundred males tested singly on celery and then on asters infected 11 celery plants and only 1 aster. Fifty lots of 5 males caused 24.0 per cent infections of celery; 50 lots of 10 males, 74 per cent; and 50 lots of 20 males, 80 per cent. Again the period of exposure of healthy celery to leafhoppers influenced virus transmission.

The percentages of infections with the second set of celery, the first and second sets of asters, show no progressive increase with lots of 5, 10, and 20 males; but lower percentages were obtained with lots of 5 than with lots of 10 and 20 males.

To Successive Asters Alternating with Celery, by Varying Numbers of Adults Reared on Infected Asters.—In the previous experiment the percentages of infection showed no progressive increase with varying numbers of leafhoppers reared on infected celery; hence another experiment was conducted with males of *Texananus latipex* reared on diseased asters. Table 4 gives the results.

TABLE 4

TRANSMISSION OF VIRUS TO SUCCESSIVE ASTERS ALTERNATING WITH CELERY, BY VARYING NUMBERS OF ADULT *Texananus latipex* REARED ON INFECTED ASTERS

Number of lots	Number of males in each lot	Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected
		First set of asters			First set of celery		
100	1	100	4	4.0	100	10	10.0
100	5	100	4	4.0	100	5	5.0
50	10	50	2	4.0	50	8	16.0
50	20	50	8	16.0	50	20	40.0
6	40	6	1	16.7	6	5	83.5
		Second set of asters			Second set of celery		
100	1	..	..	...	..	..	...
100	5	50	0	0.0	50	3	6.0
50	10	50	2	4.0	50	7	14.0
50	20	50	4	8.0	50	8	16.0
6	40	6	0	0.0	6	4	66.7

One hundred males each tested singly on healthy celery for 4 weeks, infected 10 celery plants, as compared with 11 plants in the previous experiment. In the first and second sets of celery there was a progressive increase in the percentages of infections obtained with lots of 5, 10, 20, and 40 males transferred weekly. Again there was no progressive increase in the percentages of infections with lots of varying numbers of adults in the first and second sets of asters. The number of leafhoppers, however, plays an important role in the transmission of virus to celery.

To Successive Asters by Adults Reared on Infected Asters.—In the experiment on the transmission of the virus to celery alternating with asters, no asters became infected by lots of 40 males or females of *Texananus lathropi*, or by females of *T. latipex* in weekly inoculations (table 2). In the experiment on transmission to asters alternating with celery, the percentages of infections by both species of leafhoppers in weekly inoculations varied considerably. For this reason another experiment was inaugurated in which the period of exposure on each successive aster was increased to 3 weeks with lots of 40 males or females of *T. latipex* reared on diseased asters. The results are indicated in table 5.

A total of 56 asters were inoculated by 10 lots of 40 males each, and 24 asters or 42.9 per cent were infected; and 27 of 55 asters or 49.1 per cent were infected by lots of 40 females (table 5). With both males and females, the highest percentages of infections were obtained with the first set of asters. As the results show, the period of exposure of healthy asters to infective adults affects the percentages of infections obtained.

TABLE 5

TRANSMISSION OF VIRUS TO SUCCESSIVE SETS OF ASTERS INOCULATED FOR THREE WEEKS, BY ADULT *Texananus latipex* REARED ON INFECTED ASTERS

Number of lots	Number of adults in each lot*	Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected
		First set of asters			Second set of asters		
10	40 males	10	10	100.0	10	4	40.0
10	40 females	10	8	80.0	10	6	60.0
		Third set of asters			Fourth set of asters		
10	40 males	10	5	50.0	10	3	30.0
10	40 females	10	4	40.0	10	4	40.0
		Fifth set of asters			Sixth set of asters		
10	40 males	9	0	0.0	7	2	28.6
10	40 females	8	1	12.5	7	4	57.1
		Total of all sets					
10	40 males	56	24	42.9			
10	40 females	55	27	49.1			

* Adults alive at the end of 12 to 18 weeks: males, 11–28; females, 15–35.

LATENT PERIOD OF VIRUS IN ADULTS

The literature on the so-called virus-incubation period in leafhoppers may be briefly reviewed. Smith and Boncquet (1915) were first to use the term "incubation period of the inciting agent of curly leaf" in connection with the beet leafhopper. Severin (1921) mentioned the "incubation period of the causative agent of curly leaf" in the same insect and later (1931) spoke of the "virus-incubation period." As pointed out by Bawden (1943), the term "incubation period" implies a fixed time during which the virus develops or multiplies. Storey (1939) wrote of "a delay in the development of infective ability," termed by other workers the "latent," "waiting," or "incubation period," and he coupled it with the retention of the virus by the vectors. Leach (1940) employs the term "latent period" in his textbook.

The latent period of the virus in the two species of leafhoppers was determined by using lots of 80 previously noninfective males or females, which were kept on diseased celery for 1 day. Daily transfers were made to successive healthy celery during a period of 41 days. The mortality of the adults by the end of 42 days was recorded. The results appear in table 6.

The minimum latent period of the virus in *Texananus lathropi*, using 80 males or females, was 7 and 8 days, respectively; in *T. latipex,* with 1 lot of 80 males, it was 8 days (table 6). The maximum latent period in *T. lathropi* was 33 days with 80 females; in *T. latipex,* 37 days with 80 males. Four lots of 80 adults of *T. lathropi* and 3 lots of *T. latipex* failed to transmit the virus.

TABLE 6

LATENT PERIOD OF VIRUS IN ADULTS OF TWO SPECIES OF PHLEPSIDS, ON DISEASED CELERY

Days on infected celery	Successive plants inoculated	Plants infected	Per cent infected	Days after transfer on which successive infections occurred, including initial day on infected celery	Adults alive at end of 42 days
Two lots of 80 males each, *Texananus lathropi*					
1	41	15	36.6	7, 10, 13, 14, 16, 17, 20, 21, 22, 23, 25, 28, 30, 31, 42.	38
1	41	6	14.6	9, 13, 19, 20, 30, 35	37
Four lots of 80 females each, *Texananus lathropi*					
1	41	8	19.5	8, 13, 14, 16, 19, 24, 30, 42...........	28
1	41	3	7.3	11, 12, 14...	54
1	41	3	7.3	33, 37, 42......	25
1	41	2	4.9	14, 17...............	58
Two lots of 80 males each, *Texananus latipex*					
1	41	17	41.5	8, 9, 12, 15, 16, 20, 22, 24, 26, 27, 29, 32, 36, 37; 39, 40, 41....................................	41
1	41	2	4.9	37, 39..	32

RETENTION OF VIRUS

By Varying Numbers of Adults.—To determine whether a longer period of exposure on diseased celery to varying numbers of adults is a factor in transmission of the virus, lots of 40, 20, and 10 males or females were kept on diseased celery for 8 days, and then each lot was transferred daily to healthy celery during 34 days (table 7).

As table 7 shows, two lots of 40 males and 1 lot of 20 males of *Texananus lathropi* produced 3 infections and 1 lot of 40 males caused 2 infections. Single infections occurred with 13 lots of 40, 20, and 10 adults of *T. lathropi* and 4 lots of 40 males of *T. latipex.* Evidently the number of leafhoppers kept on diseased celery for 8 days, and then transferred daily to healthy celery during a period of 34 days, determines the number of infections obtained. Seven lots of 40, 9 lots of 20, and 11 lots of 10 adults of *T. lathropi* and 6 lots of 40 and 4 lots of 20 males of *T. latipex* failed to cause infection.

Since 7 lots of 80 adults of *Texananus lathropi* produced 2 to 15 infections

TABLE 7

Retention of Virus by Varying Numbers of Adult *Texananus lathropi* and *T. latipex*

Days on infected celery	Successive plants inoculated	Plants infected	Per cent infected	Days after transfer on which successive infections occurred including 8 days on infected celery	Adults alive at end of 42 days
				Five lots of 40 males each, *Texananus lathropi*	
8	34	3	8.8	10, 13, 31	35
8	34	3	8.8	21, 26, 31	18
8	34	2	5.9	39, 42	19
8	34	1	2.9	16	19
8	34	1	2.9	20	30
				Four lots of 40 females each, *Texananus lathropi*	
8	34	1	2.9	15	14
8	34	1	2.9	20	28
8	34	1	2.9	30	33
8	34	1	2.9	36	28
				Three lots of 20 males each, *Texananus lathropi*	
8	34	3	8.8	36, 41, 42	13
8	34	1	2.9	38	16
8	34	1	2.9	42	10
				Two lots of 20 females each, *Texananus lathropi*	
8	34	1	2.9	35	17
8	34	1	2.9	41	15
				Two lots of 10 males each, *Texananus lathropi*	
8	34	1	2.9	26	9
8	34	1	2.9	40	6
				One lot of 10 females, *Texananus lathropi*	
8	34	1	2.9	34	6
				Three lots of 40 males each, *Texananus latipex*	
8	34	1	2.9	13	29
8	34	1	2.9	34	20
8	34	1	2.9	40	26
				One lot of 20 males, *Texananus latipex*	
8	34	1	2.9	17	16

(table 6) or an average of 5.3, in determining the latent period of the virus, it seemed desirable to increase the time spent on diseased celery. Two lots of 80 female *T. lathropi,* after completing the nymphal stages on infected celery, an average of 66.4 days (table 10), were provided with healthy celery for 41 days. Each of the 2 lots caused 23 infections, or 56.1 per cent. Again it is evident that the amount of virus obtained by the nymphs in a long period on diseased celery, and the number of adults used, influences the number of infections produced.

By Single Adults.—The retention of the virus was determined by single males and females of both species of leafhoppers, all of which completed the nymphal stages on infected celery. Each leafhopper was confined in a cage enclosing a healthy celery plant until symptoms developed and then was transferred daily to successive healthy celery plants during adult life. The results are indicated in table 8.

The virus was retained 5 to 77 days (males) or 62 days (females) by *Texananus lathropi;* 22 to 39 days (males) and 1 to 42 days (females) by *T. latipex.* Two adults of *T. lathropi* and 10 of *T. latipex* caused only 1 infection each. The period on the first plant is not included in the retention of the virus, since the adults were able to acquire the virus again.

ATTEMPTS TO TRANSMIT VIRUSES OF CURLY TOP AND PIERCE'S DISEASE OF GRAPEVINE

Because of the interest in two other prevalent viruses, attempts were made to transmit these by means of the two species of leafhoppers.

Fifty noninfective female *Texananus lathropi* and 50 noninfective male *T. latipex* were kept on curly-top beets for 2 days after which each lot was transferred to 10 successive healthy sugar beets. All beets remained healthy. At the end of 12 days, 43 females of *T. lathropi* and 40 males of *T. latipex* were alive.

Texananus lathropi failed to transmit the virus of Pierce's disease of grapevine to 10 healthy grapevines and 10 plants of common or Chilean alfalfa, *Medicago sativa* L.; and from alfalfa dwarf to 10 healthy grapevines and 10 alfalfa plants. *T. latipex* also failed to transmit that same virus to 5 healthy grapevines and 5 alfalfa plants and from alfalfa dwarf to 10 healthy grapevines and 5 alfalfa plants.

BIOLOGY OF TEXANANUS LATHROPI AND T. LATIPEX

DeLong and Severin (1945) have described the characters of the male and female *Texananus lathropi* and *T. latipex.* These closely related species can be readily distinguished by the U-shaped excavation (plate 1, *A*) which extends halfway through the last ventral segment of the female *T. lathropi* and by the V-shaped indentation (plate 1, *B*) of the posterior margin of the last sternite of *T. latipex.*

According to Osborn and Lathrop (1923), very few species of *Phlepsius* have been studied for life history; there is an opportunity for careful work in determining detail of habits, life history, and ecology.

Oviposition.—The eggs of *Texananus lathropi* are deposited in two rows in the petioles of celery (plate 1, *C*) or in the stems of aster plants. One egg of

TABLE 8

Retention of Virus by Single Adults of Two Species of Phlepsids with Celery as the Host Plant

Days on first plant before symptoms developed	Plants inoculated after first infection	Plants infected after first infection	Per cent infected after first infection	Days after first infection on which successive infections occurred	Longevity of adults, days
Six lots of single males, *Texananus lathropi*					
28	77	12	15.6	37, 39, 41, 44, 47, 53, 55, 57, 58, 58, 68, 72, 77	105
29	24	6	25.0	4, 5, 6, 15, 17, 21	53
28	11	2	1.8	4, 5	39
29	80	1	1.2	72	109
29	86	1	1.2	10	115
28	79	0	0.0	0	107
Two lots of single females, *Texananus lathropi*					
26	56	1	1.8	62	82
34	95	0	0.0	0	129
Eleven lots of single males, *Texananus latipex*					
30	98	5	5.1	26, 27, 28, 36, 39	128
24	34	4	11.8	14, 32, 33, 34	58
28	28	1	3.6	28	56
23	76	1	1.3	22	99
30	80	0	0.0	0	110
30	59	0	0.0	0	89
40	57	0	0.0	0	97
24	41	0	0.0	0	65
28	41	0	0.0	0	69
30	19	0	0.0	0	49
26	15	0	0.0	0	41
Nine lots of single females, *Texananus latipex*					
25	44	8	18.2	8, 10, 14, 23, 25, 29, 33, 37	69
32	103	7	9.6	2, 5, 19, 20, 30, 40, 42	135
36	29	1	3.4	29	65
33	28	1	3.6	14	61
45	2	1	50.0	2	47
21	1	1	100.0	1	22
44	70	0	0.0	0	114
42	51	0	0.0	0	93
42	51	0	0.0	0	93

one row is deposited between two eggs of the opposite row (plate 1, *D*). The petiole sometimes cracks when long rows of eggs are deposited. After inserting the egg in the petiole, the female secrets a liquid (which becomes white when dry) in the form of a papilla over each egg puncture. Sometimes the papilla terminates in a hairlike structure. The papillae covering two eggs in opposite rows are arranged in V shape. In hatching, the nymph often pushes off the papilla. After all the nymphs have emerged from two rows of eggs, the zigzag arrangement of the openings of the egg punctures can readily be seen with a binocular microscope. The papilla is a secretion from the accessory glands and probably protects the eggs from parasites.

The eggs of *Texananus latipex* are deposited singly or in one short row. A quantity of accessory gland secretion covers the egg puncture and may be flattened against the petiole; or the papilla may be upright or vertical, terminating in a hair.

TABLE 9

DURATION OF EGG PERIODS AND DAILY EMERGENCE OF NYMPHS OF TWO SPECIES OF PHLEPSIDS

Days from oviposition to hatching	Eggs deposited January 15–22; temperature: 72.8°-78.9°; mean 68.4° F		Eggs deposited February 15-19; temperature: 68.5°–77.1°; mean 60.5° F		Eggs deposited March 16-20; temperature: 67.6°-76.5°; mean 59° F		Eggs deposited April 16–19: temperature; 69.7°–79.7°; mean 60° F	
	Nymphs hatched	Percentage of total hatched	Nymphs hatched	Percentage of total hatched	Nymphs hatched	Percentage of total hatched	Nymphs hatched	Percentage of total hatched
				Texananus lathropi				
19	..	....	65	5.2	34	19.0	1	2.3
20	4	1.7	360	29.0	71	39.7	5	11.6
21	68	29.3	375	30.0	45	25.1	17	39.5
22	62	26.7	241	19.4	24	13.4	13	30.2
23	60	25.9	153	12.3	4	2.2	7	16.3
24	22	9.5	35	2.8	1	0.6		....
25	13	5.6	8	0.6	..	....	..	....
26	3	1.3	3	0.2	..	....	..	....
Total	232		1,240		179		43	
				Texananus latipex				
19	..	....	..	....	29	27.4	..	....
20	3	13.0	17	31.5	41	38.7	2	11.1
21	3	13.0	25	46.3	22	20.8	11	61.1
22	7	30.0	11	20.4	9	8.5	3	16.7
23	8	34.8	1	1.9	5	4.7	2	11.1
24	1	4.3	..	....	..	....	..	....
25	1	4.3	..	....	..	....	..	....
Total	23		54		106		18	

Egg.—The average length of eggs deposited by the two species of leafhoppers is 1.05 mm; the average width 0.28 mm. The eggs are translucent when first deposited. They are elongated and are narrower at one end than at the other. Near the hatching period, the compound eyes of the embryo become visible.

Egg Periods.—The egg periods of each species of leafhopper were determined from eggs deposited during 4 months from January to April, inclusive. Females of each species were confined in cages enclosing potted celery plants and were transferred daily to successive plants during a period of 3 to 7 days. The plants in which the eggs were deposited were covered with cages and ob-

TABLE 10

DURATION OF PERIODS BETWEEN MOLTS OF TWO SPECIES OF PHLEPSIDS

Sex and date hatched	Duration of stadia, days					
	First instar	Second instar	Third instar	Fourth instar	Fifth instar	Total
			Texananus lathropi			
Males:						
January 20	11	8	9	11	20	59
January 30	13	7	8	9	19	56
January 30	12	9	9	11	21	62
February 1	11	8	9	11	22	61
Average	*11.7*	*8.0*	*8.7*	*10.5*	*20.5*	*59.5*
Females:						
February 1	11	9	9	12	22	63
February 10	12	9	9	14	20	64
February 10	9	10	10	14	21	65
February 10	12	11	12	13	20	68
February 10	14	10	12	15	21	72
Average	*11.6*	*9.8*	*10.4*	*13.6*	*20.8*	*66.4*
			Texananus latipex			
Males:						
February 4	10	9	8	10	19	56
February 11	13	9	11	12	14	59
March 5	15	10	6	8	13	52
March 6	12	9	6	7	13	47
March 7	12	10	7	8	12	49
Average	*12.4*	*9.4*	*7.6*	*9.0*	*14.2*	*52.6*
Females:						
February 4	10	9	8	10	19	56
February 4	10	11	9	11	15	56
February 4	11	10	10	12	17	60
February 7	12	12	11	15	23	73
February 9	12	9	9	12	18	60
February 11	13	9	11	12	14	59
February 11	12	9	10	13	17	61
March 4	7	11	13	9	14	54
March 5	10	8	10	9	17	54
March 5	13	12	6	12	15	55
March 6	12	9	6	8	15	50
March 7	9	10	7	8	11	45
March 7	10	10	7	9	13	49
Average	*10.1*	*9.9*	*9.0*	*13.0*	*16.0*	*56.3*

served daily for the hatching of nymphs. Eggs deposited during one day usually hatched over a period of several days. In an effort to measure the egg periods more accurately, a daily count of the nymphs hatched in each cage was taken, and the results of each monthly test were summed up and tabulated. The egg periods, with the number and percentage of nymphs that hatched, are indicated in table 9.

As this table shows, the egg periods of *Texananus lathropi* were longer with eggs deposited in January and February than with those laid in March and April. In *T. latipex* the periods were longer with eggs deposited in January than with those dating from February, March, and April. *T. lathropi* produced more eggs than *T. latipex*, though approximately equal numbers of females were used. Conceivably, a higher percentage of the *T. lathropi* may have been at the egg-laying stage.

Period between Molts.—The interval or period between molts (stages or stadia) and the total duration of the nymphal stages of both species of leafhoppers is given in table 10. The males of *Texananus lathropi* required 56 to 62 days, an average of 59.5, to complete the nymphal stages; the females 63 to 72 days, an average of 66.4. To reach the adult stage, the males of *T. latipex* required 47 to 59 days, an average of 52.6; the females 45 to 73 days, an average of 56.3. With both species of leafhoppers, the total duration of the nymphal stages is shorter for males than for females.

TABLE 11

AVERAGE MEASUREMENTS IN MILLIMETERS OF TWO SPECIES OF PHLEPSIDS AFTER EACH MOLT

Nymphs and adults	Diameter, head across compound eyes		Length, head, thorax, and abdomen		Length, head to end of wings	
	Range	Mean	Range	Mean	Range	Mean
			Texananus lathropi			
First instar	0.40-0.43	0.41	1.15-1.32	1.24	....	....
Second instar	0.53-0.57	0.55	1.62-2.16	1.81	....	....
Third instar	0.73-0.76	0.75	2.43-2.80	2.60	....	....
Fourth instar	0.94-1.03	0.99	2.97-3.40	3.16	....	....
Fifth instar	1.27-1.36	1.31	3.73-4.34	4.03	....	....
Adult male	1.39-1.44	1.42	4.43-5.41	5.02	4.59-5.20	4.97
Adult female	1.46-1.55	1.51	4.94-5.99	5.43	5.08-5.48	5.26
			Texananus latipex			
First instar	0.39-0.40	0.40	1.15-1.38	1.25	....	....
Second instar	0.53-0.55	0.54	1.59-1.89	1.73	....	....
Third instar	0.66-0.71	0.69	1.89-2.48	2.21	....	....
Fourth instar	0.89-0.96	0.92	2.67-3.32	2.84	....	....
Fifth instar	1.17-1.31	1.24	3.43-4.10	3.80	....	....
Adult male	1.34-1.49	1.40	3.89-5.18	4.68	4.37-5.02	4.79
Adult female	1.35-1.51	1.45	4.43-5.55	4.99	4.78-5.35	5.00

Measurements.—Table 11 gives the average measurements for various parts of the body of ten specimens one day after each molt, together with measurements for the adults of both leafhopper species. As shown by the mean measurements, the nymphal instars, males, and females of *Texananus lathropi* are larger than those of *T. latipex*. The males of the two species are smaller than the females. The instars can be determined accurately from the measurements of the head across the compound eyes.

Color of Nymphal Instars.—Recently hatched nymphs of both species are white with pink compound eyes. A few hours after hatching, the nymphs of both species become dark brown or black, with light areas of brown, yellow, and white that give them a mottled appearance (plate 2, *A* to *F*). The color patterns of the five instars of both species are almost identical; but, in general, the nymphs of *Texananus latipex* are darker than those of *T. lathropi.*

Color of Adults.—Adults of *Texananus lathropi* have a slight rosaceous tinge, a little more distinct on the elytrae than on the head and pronotum. Osborn and Lathrop (1923), who described the species, discuss the color pattern in detail. Two patterns occur with the males and females of *T. latipex*—dark-brown and light-brown forms, mottled with white and black (plate 2, *G, H, I, J*); details are given by DeLong (1943).

Mating.—An attempt was made to mate female *Texananus lathropi* with male *P. latipex* after the last molt, and female *T. latipex* with male *T. lathropi.* The experiment was repeated with 10 females of each species. Coition did not occur. Though eggs were deposited by some females, not a single nymph hatched. Evidently the two species will not cross or interbreed.

Preoviposition Period.—The period from the time the female reached the winged stage after the last molt until egg laying began was determined with each species. The preoviposition period of 2 females of *Tenananus lathropi* that passed through the last molt on January 17 and 20, was 40 and 44 days respectively. Two females that reached the winged stage on April 16 required 20 and 34 days. The preoviposition period of *T. latipex* that passed through the last molt on May 2 was 34 days; May 4, 16 days; and May 6, 23 days.

Egg-laying Capacity.—To determine how many eggs a single female of *Texananus lathropi* deposits during her adult life, 1 female and 1 to 5 males after the last molt were confined in each cage enclosing a large healthy celery plant. When the celery became unfavorable as food, the female and males were transferred to a second plant—sometimes to a third. The eggs were allowed to hatch; and the total number of nymphs removed from each cage would equal the egg-laying capacity, provided all the eggs hatched. The nymphs were removed twice a month from each cage. The numbers that hatched from eggs deposited by each of 5 females were as follows: 229, 231, 345, 369, and 395, or an average of 341. The numbers that hatched from eggs deposited by each of 4 female *T. latipex* were as follows: 140, 171, 180, and 208, or an average of 175.

VIRUS TRANSMISSION EXPERIMENTS WITH *TEXANANUS SPATULATUS* VAN DUZEE

(Plate 3, *C, D*)

A study was undertaken on the possibility of transmitting the virus during the nymphal stages. To determine the efficiency of the vector, virus-transmission experiments were conducted with single males and females; varying numbers of adults were tested on healthy celery and asters, and upon successive asters. Attempts were made to determine the latent period of the virus in the adults, and the ability of this insect to transmit the viruses of curly top and Pierce's disease of grapevine.

The characters, distribution, and food plants of *Texananus spatulatus* have already been discussed by DeLong and Severin (1945).

EXPERIMENTAL METHODS

In the transmission studies of the California aster-yellows virus, adults were used that had completed the nymphal stages on infected celery, and sometimes on asters. If leafhoppers were not reared, then the adults were fed on diseased celery for 10 days or longer to complete the latent period of the virus. Noninfective *Texananus spatulatus* were obtained by allowing females to oviposit in healthy sugar beets, immune to aster yellows; and the nymphs that hatched were transferred to healthy celery. Because sugar beets are not a favorable food plant of this insect, many nymphs failed to reach the adult stage.

TABLE 12

TRANSMISSION OF VIRUS TO CELERY BY VARYING NUMBERS OF NYMPHS OF *Texananus spatulatus*

Number of lots	Number of nymphs in each lot	Days on infected celery	Days on healthy celery	Celery		
				Plants inoculated	Plants infected	Per cent infected
			Stadium I			
3	5	3	3	3	0	0.0
2	5	4	4	2	0	0.0
3	10	3	3	3	0	0.0
2	10	4	4	2	0	0.0
			Stadium II			
2	5	11	6	2	2	0.0
3	5	12	7	3	3	0.0
2	10	11	6	2	2	100.0
3	10	12	7	3	3	100.0
2	20	10	6	2	2	100.0
3	20	11	7	3	3	100.0
4	20	12	8	4	4	100.0
			Stadium III			
2	5	20	10	2	2	100.0
3	10	20	10	3	3	100.0
2	20	20	10	2	2	100.0
			Stadium IV			
2	5	30	30	2	2	100.0
1	10	30	30	1	1	100.0
1	20	30	30	1	1	100.0

TRANSMISSION OF VIRUS TO CELERY

By Varying Numbers of Nymphal Instars.—An experiment was conducted to determine whether each nymphal instar could transmit the virus. Lots consisting of varying numbers of each instar were fed during varying periods on infected and healthy celery. As the results show (table 12), the first instar was not able to transmit the virus, whereas each lot of the second (with 2 exceptions), third, and fourth instars infected healthy plants.

By Single Males and Females.—A comparison was made of the transmission of the virus by single male and female leafhoppers. After the adults had remained on diseased celery for 30 days, each leafhopper was fed on healthy celery until the plant manifested symptoms or until the insect died. As table 13 shows, 34 of 100 males and 29 of 100 females transmitted the virus to healthy plants.

By Varying Numbers of Adults.—A comparison was made of the infections produced by 5 males and 5 females that had completed the nymphal stages on diseased celery. Each lot of adults was transferred to a healthy celery plant and kept there until symptoms developed; then the surviving adults were transferred to a second healthy celery plant.

TABLE 13

Transmission of Virus to Celery by Varying Numbers of Adult *Texananus spatulatus*

Number of lots	Number of adults in in each lot	First set of celery			Second set of celery		
		Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected
100	1 male	100	34	34 0			
100	1 female	100	29	29 0			
25	5 males	25	22	88 0	15	6	40 0
25	5 females	25	18	72 0	8	4	50 0

Table 13 shows the results obtained The virus was transmitted to the first set of healthy celery by 22 of 25 lots of 5 males, or 88 0 per cent; and to 6 of 15 of the second set of plants, or 40 per cent It was transmitted to the first set of celery by 18 of 25 lots of 5 females, or 72 0 per cent; and to 4 of 8 plants of the second set, or 50 0 per cent

TRANSMISSION OF VIRUS TO ASTERS ALTERNATING WITH CELERY

A comparison was made of the transmission of the virus to healthy asters and celery. Five lots of 40 males and 5 lots of 40 females, which had completed the nymphal stages on infected celery, were transferred daily or weekly to successive healthy asters alternating with celery (table 14).

The percentages of celery plants infected were as follows: daily, by males 28.7 per cent, by females 17.3 per cent; weekly, by males 80.0 per cent, by females 23.1 per cent. Since lower percentages of infections were obtained in daily than in weekly transfers, evidently the period of exposure of the leafhoppers on healthy celery is a factor in virus transmission. In daily and weekly transfers of the males and females, only 1 aster was infected, and that was by daily transfers of the males (table 14).

TRANSMISSION OF VIRUS TO ASTERS

In the experiment on the transmission of the virus to asters alternating with celery in daily and weekly transfers, only 1 aster was infected of 188 inoculated (table 14). For this reason, the period of exposure on each aster was increased. Fifty lots of 5 males or 5 females that had completed the nymphal stages on diseased celery were transferred and kept on healthy aster until they died. Two of 25 asters, or 8.0 per cent, were infected by lots of 5 males; 3 of 25 asters, or 12 per cent, by lots of 5 females (table 15). Possibly if the adult life on asters had been longer, a higher percentage of infection would have resulted.

TABLE 14

TRANSMISSION OF VIRUS TO ASTERS ALTERNATING WITH CELERY, BY ADULT *Texananus spatulatus*

Number of lots	Number of adults in each lot	Asters			Celery		
		Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected
	Daily inoculations						
5	40 males	79	1	1.3	80	23	28.7
5	40 females	81	0	0.0	81	14	17.3
	Weekly inoculations						
5	40 males	15	0	0.0	15	12	80 0
5	40 females	13	0	0.0	13	3	23 1

TABLE 15

TRANSMISSION OF VIRUS TO ASTERS BY VARYING NUMBERS OF ADULT *Texananus spatulatus*

Number of lots	Number of adults in each lot	Plants inoculated	Plants infected	Per cent infected
50	1 male	50	0	0.0
50	1 female	50	0	0.0
25	5 males	25	2	8.0
25	5 females	25	3	12 0
5	40 males	59	1	1.7
5	40 females	150	1	0.7

It was decided to rear the leafhoppers on diseased asters instead of celery. Ten lots of 40 males or females were transferred daily to successive healthy asters during adult life. One of 59 asters, or 1.7 per cent, was infected by the males; and 1 of 150 asters, or 0.7 per cent, by the females (table 15).

Fifty recently molted males and 50 females reared on infected asters were transferred singly to healthy asters. No aster yellows developed.

LATENT PERIOD OF VIRUS IN ADULTS

The latent period of the virus was determined with lots of 40 males or females. After 1 day on infected celery, the adults were transferred daily to successive healthy celery plants throughout a period of 41 days. The mortality of the adults at the end of 42 days was recorded (table 16). The minimum latent period of the virus was 6 days; the maximum, 35 days. Only 18 of the 410 celery plants inoculated, or 4.4 per cent, became infected. This low percentage can be explained by the fact that each healthy plant was exposed to the adults for 1 day. One lot of males and 1 lot of females failed to transmit the virus.

TABLE 16

LATENT PERIOD OF VIRUS IN ADULTS OF *Texananus spatulatus* WITH CELERY AS THE HOST PLANT

Days on infected celery	Successive plants inoculated	Plants infected	Per cent infected	Days after transfer on which successive infections occurred, including initial day on infected celery	Adults alive at end of 42 days
Four lots of 40 males each					
1	41	3	7.3	6, 19, 35	29
1	41	3	7.3	12, 14, 15	16
1	41	1	2.4	33	21
1	41	1	2.4	35	37
Four lots of 40 females each					
1	41	3	7.3	17, 18, 29	22
1	41	3	7.3	23, 41, 42	26
1	41	2	4.9	16, 19	30
1	41	2	4.9	18, 27	11

RETENTION OF VIRUS

By Varying Numbers of Adults.—To determine whether a longer period of exposure on diseased celery is a factor in transmission of the virus by varying numbers of leafhoppers, lots of 20, 10, and 5 adults were kept on infected celery for 8 days and then were transferred daily to healthy celery for 34 days (table 17). Nine lots of 20 males and females infected 23 of 306 plants inoculated, or 7.5 per cent; 9 lots of 10 males and females infected 39 of 306 plants, or 12.4 per cent; and 4 lots of 5 males infected 4 of 136 plants, or 2.9 per cent. The latent period of the virus with lots of 20, 10, and 5 adults cannot be determined accurately: since the insects were kept on diseased celery for 8 days, it is not known whether they picked up the virus on the first day or later.

By Single Adults.—The retention of the virus was determined by use of single males and females, each of which had completed the nymphal stages on diseased celery. Each leafhopper was kept on a healthy celery plant until the latter showed symptoms; then the insect was transferred daily to successive healthy celery plants throughout its adult life. Table 18 shows the results.

One male retained the virus for 84 days, and 1 female for 99 days. Seven males and 6 females produced only 1 infection. The first infection is not included in the retention of the virus, since the adults were able to pick up the virus again.

TABLE 17

RETENTION OF VIRUS BY VARYING NUMBERS OF ADULT *Texananus spatulatus*

Number of adults in each lot	Days on infected celery	Successive plants inoculated	Plants infected	Per cent infected	Days after transfer on which successive infections occurred, including initial day on infected celery	Adults alive at end of 42 days
Five lots of 20 males each						
20	8	34	5	14.7	37, 39, 40, 41, 42	14
20	8	34	3	8.8	9, 11, 15	6
20	8	34	1	3 0	13	9
20	8	34	1	3.0	25	7
20	8	34	1	3.0	39	9
Four lots of 20 females each						
20	8	34	6	17.6	16, 24, 26, 32, 34, 36	9
20	8	34	3	8.8	11, 12, 15	5
20	8	34	2	5.9	12, 41	17
20	8	34	1	3 0	31	13
Five lots of 10 males each						
10	8	34	12	35.3	10, 11, 17, 23, 24, 29, 30, 33, 34, 37, 39, 42	8
10	8	34	7	20.6	14, 30, 31, 32, 34, 38, 40	10
10	8	34	5	14.7	9, 10, 38, 40, 41	8
10	8	34	4	11.8	22, 23, 33, 35	4
10	8	34	3	8.8	25, 28, 35	6
Four lots of 10 females each						
10	8	34	4	11.8	28, 35, 38, 40	9
10	8	34	2	5.9	31, 41	6
10	8	34	1	3.0	38	6
10	8	34	1	3.0	39	5
Four lots of 5 males each						
5	8	34	1	3.0	9	4
5	8	34	1	3.0	30	3
5	8	34	1	3.0	32	3
5	8	34	1	3.0	39	5

ATTEMPTS TO TRANSMIT VIRUSES OF CURLY TOP AND PIERCE'S DISEASES OF GRAPEVINE

An attempt was made to transmit the curly-top virus by means of *Texananus spatulatus.* Fifteen lots of 5 to 20 males or females reared during the nymphal stages on nettleleaf goosefoot (sowbane), *Chenopodium murale,* were exposed to curly-top beets for periods varying from 2 to 14 days; and then each lot was transferred to a healthy beet. All beets remained healthy.

An attempt was made to transmit and recover the curly-top virus from susceptible host plants on which *Texananus spatulatus* were collected in the field. Noninfective leafhoppers after feeding on 2 nettleleaf goosefoot plants and 2 rough pigweeds, *Amaranthus retroflexus,* inoculated by infective beet leafhoppers, failed to recover the virus and transmit it to healthy sugar beets.

In another test 135 adults collected on pasture vegetation on the summit of Pacheco Pass on May 5, 1942, were distributed on 3 curly-top beets and kept there for 2 days; then each lot was transferred to a healthy sugar beet. All beets remained healthy.

TABLE 18

RETENTION OF VIRUS BY SINGLE ADULTS OF *Texananus spatulatus* WITH CELERY AS THE HOST PLANT

Days on first plant before symptoms developed	Plants inoculated after first infection	Plants infected after first infection	Per cent infected after first infection	Days after first infection on which successive infections occurred	Longevity of adults, days
			Ten lots of single males		
39	105	6	5.7	14, 17, 30, 37, 38, 39	144
46	95	4	4.2	26, 54, 63, 84	141
46	36	2	5.6	14, 27	82
34	98	0	0.0		132
39	21	0	0.0		60
26	14	0	0.0		40
41	12	0	0.0		53
27	50	0	0.0		77
31	4	0	0.0		35
23	2	0	0.0		25
			Eight lots of single females		
31	169	4	2.3	45, 62, 79, 99	200
35	95	3	3.2	26, 35, 60	130
28	131	0	0.0		159
45	102	0	0.0		147
37	35	0	0.0		72
31	12	0	0.0		43
34	4	0	0.0		38
27	1	0	0.0		28

Lots of 20 adults of *Texananus spatulatus,* after feeding on Pierce's disease of grapevines, failed to transmit the virus to 6 healthy grapevines and 5 common or Chilean alfalfa plants, and from alfalfa dwarf to 5 healthy grapevines and to 5 alfalfa plants.

There was no evidence to show that the beet leafhopper, *Eutettix tenellus* (Bakers), could transmit the curly-top virus to healthy grapevines. Severin (1928) demonstrated that Hairy Peruvian alfalfa, *Medicago sativa* L., was naturally and has been experimentally infected with curly top. The beet leafhopper failed to transmit the virus of Pierce's disease of grapevine to healthy grapevines and alfalfa.

TEXANANUS PERGRADUS DELONG AS A VECTOR

(Plate 3, *E, F*)

This phlepsid leafhopper has been collected on a variety of food plants, as shown by DeLong and Severin (1945). It has been reared on celery infected with aster yellows, but few nymphs reached the adult stage.

Adults that completed the nymphal stages on infected celery were transferred singly to healthy celery. If symptoms of the disease developed, the insects were fed on one or more additional plants. Single males and females each transferred to one or more healthy celery plants transmitted the virus to 5 of 14 and 11 of 22 plants respectively (table 19). In other tests, adults collected in the field were kept on infected celery for 10 days or longer to complete the latent period of the virus, before transferring to healthy plants. Lots of 2 to 10 adults infected 12 of 31 plants inoculated as shown in table 19.

TABLE 19

TRANSMISSION OF VIRUS TO CELERY BY VARYING NUMBERS OF ADULT *Texananus oregonus*

Males					Females				
Number of lots	Number of adults in each lot	Plants inoculated	Plants infected	Per cent infected	Number of lots	Number of adults in each lot	Plants inoculated	Plants infected	Per cent infected
8	1	14	5	35.7	11	1	22	11	50.0
1	2	7	1	14.3	1	2	12	2	16.7
1	3	1	1	100.0	2	3	2	1	50.0
4	10	4	4	100.0	2	5	4	2	50.0
..	..	..	..	.....	1	10	1	1	100.0

TEXANANUS PERGRADUS DELONG AS A VECTOR

(Plate 3, *G, H*)

This leafhopper was reared on celery infected with aster yellows from 12 males and 14 females collected on May 4, 1940, on pasture vegetation along a riverbank at Woodlake, Tulare County. The characters, distribution, and food plants are reported by DeLong and Severin (1945).

Fifty males and 50 females tested singly on healthy celery failed to cause infection, but 1 aster was infected by 1 of 50 males as is shown in table 20. Twelve lots of 10, 25 lots of 20, and 5 lots of 40 males produced 1, 5, and 1 infections as indicated in table 20.

PHLEPSIUS APERTINUS OSBORN AND LATHROP AS A VECTOR

(Plate 3, *A, B*)

This leafhopper is common in alfalfa fields. The characters, distribution, and food plants have been discussed by DeLong and Severin (1945). The insect has not been reared on any host plant up to the present time.

Adults collected in the field were kept on diseased celery for 10 days or

TABLE 20

TRANSMISSION OF VIRUS TO CELERY AND ASTERS BY VARYING NUMBERS OF ADULT *Texananus pergradus*

Males					Females				
Number of lots	Number of adults in each lot	Plants inoculated	Plants infected	Per cent infected	Number of lots	Number of adults in each lot	Plants inoculated	Plants infected	Per cent infected
Celery									
50	1	50	0	0.0	50	1	50	0	0.0
12	5	12	0	0.0	2	5	2	0	0.0
12	10	12	1	8.3	1	10	1	0	0.0
25	20	25	5	20.0	3	20	3	0	0.0
5	40	48	1	2.1	5	40	45	0	0.0
Asters									
2	40	50	1	2.0	2	40	60	0	0.0

TABLE 21

TRANSMISSION OF VIRUS TO CELERY BY VARYING NUMBERS OF ADULT *Phlepsius apertinus*

Males					Females				
Number of lots	Number of adults in each lot	Plants inoculated	Plants infected	Per cent infected	Number of lots	Number of adults in each lot	Plants inoculated	Plants infected	Per cent infected
9	1	13	7	53.8	10	1	14	8	57.1
1	2	1	0	0.0	1	5	2	0	0.0
1	5	1	0	0.0	1	6	2	1	50.0
1	9	1	1	100.0	9	20	12	5	41.7
1	10	1	1	100.0	1	25	5	1	20.0
1	12	2	2	100.0	1	30	1	1	100.0
1	13	2	0	0.0	..	..	..	..	.....
1	14	3	2	66.7	..	..	..	..	.....
1	15	1	0	0.0	..	..	..	..	.....
3	20	3	0	0.0	..	..	..	..	.....

longer to complete the latent period of the virus. Males and females tested singly on healthy celery plants transmitted the virus to 7 of 13, and 8 of 14 plants respectively. Lots of 2 to 30 adults produced 14 infections as shown in table 21.

SUMMARY

Six newly demonstrated vectors of the California aster-yellows virus are reported in this paper; this proves that the virus is not transmitted by a specific leafhopper.

Texananus lathropi and *T. latipex:* The infections produced by 150 males and 150 females of *T. lathropi*, each kept singly on a healthy celery plant, was 18.7 and 16.0 per cent respectively, and by 100 males and 100 females of *T. latipex* was 18.0 and 32 per cent respectively.

Lots of 40 males or females of both leafhopper species, placed on celery alternating with asters, caused lower percentages of infections in celery in daily than in weekly transfers, a fact indicating that the period of exposure of healthy celery to the leafhoppers affects virus transmission. Lower percentages of infections occurred with asters than with celery; and, with one exception, lower percentages of asters were infected in daily than in weekly transfers. Higher percentages of infections occurred with 6 successive asters inoculated by *Texananus latipex* every 3 weeks than in daily inoculations or, with one exception, weekly inoculations—a fact again demonstrating that the period of exposure of asters to leafhoppers influences virus transmission.

Since there was an increase in the percentages of infections in celery with lots of 5, 10, and 20 adults, apparently the number of leafhoppers play a role in virus transmission.

The minimum latent period of the virus in the males and females of *Texananus lathropi* was 7 and 8 days respectively; in *T. latipex* with 1 lot of 80 males it was 8 days. The maximum latent period in *T. lathropi* was 33 days; in *T. latipex,* 37 days.

The virus was retained by single adults of *Texananus lathropi* from 5 to 77 days and by *T. latipex* from 1 to 42 days after the first infection was produced. Many of the leafhoppers of both species caused only 1 infection each.

Attempts to transmit the viruses of curly top and Pierce's disease of grapevine by means of the two species of leafhoppers were failures.

Life history studies were made of the egg periods, egg-laying capacity, and duration of the nymphal instars.

Texananus spatulatus: The aster-yellows virus was not transmitted by the first nymphal instar; probably the latent period of the virus was not completed. All other nymphal instars transmitted the virus.

Single males infected 34 per cent of the celery, and single females 29 per cent. Lots of 5 males transmitted the virus to 88.00 per cent and lots of 5 females to 72.0 per cent of the first set of celery and to 40.0 and 50.0 per cent of the second set respectively.

Lots of 40 males and 40 females infected 28.7 and 17.3 per cent respectively in daily transfers to successive celery plants, and 80.0 and 23.1 per cent in weekly transfers. Only 0.6 per cent of the asters were infected in daily transfers and none in weekly transfers to successive asters.

Lots of 5 males and 5 females kept on asters during adult life infected 8.0 and 12.0 per cent of the asters respectively. Lots of 40 males and 40 females reared on diseased asters infected 1.7 and 0.7 per cent, respectively, in daily transfers to successive asters.

The latent period of the virus in the adults was 6 to 42 days.

One male retained the virus for 84 days, and one female 99 days. Most adults caused one infection and then apparently lost the infective dose.

All attempts to transmit the curly-top virus by means of *Texananus spatulatus* were failures. The leafhopper did not recover the virus from susceptible host plants on which they were collected in the field, such as *Chenopodium murale* and *Amaranthus retroflexus,* and transmit it to healthy sugar beets. This leafhopper failed to transmit the virus of Pierce's disease of grapevine and alfalfa dwarf to healthy grapevine seedlings and alfalfa.

There was no evidence that the beet leafhopper could transmit the curly-top virus to healthy grapevine seedlings or the virus of Pierce's disease of grapevine to healthy grapevines and alfalfa.

Texananus oregonus: Males and females tested singly on healthy celery infected 5 of 14 plants and 11 of 22 plants respectively. Lots of 2 to 10 adults infected 18 to 34 plants inoculated.

Texananus pergradus: 100 males and females kept singly on healthy celery caused no infections, but 1 aster was infected by 1 of 50 males. Twelve lots of 10, 25 lots of 20, and 5 lots of 40 males produced 1, 5, and 1 infection.

Phlepsius apertinus: With this species, 13 males and 12 females infected 7 of 13 and 8 of 14 celery plants respectively. Lots of 2 to 30 adults produced 14 infections.

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Thanks are due to J. S. Lilley, who did part of the preliminary work on the life histories of *Texananus lathropi* and *T. latipex.* Mr. Lilley also conducted some of the experiments on daily and weekly transfers of the leafhoppers to celery and asters, and on the retention of the virus.

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PLATES

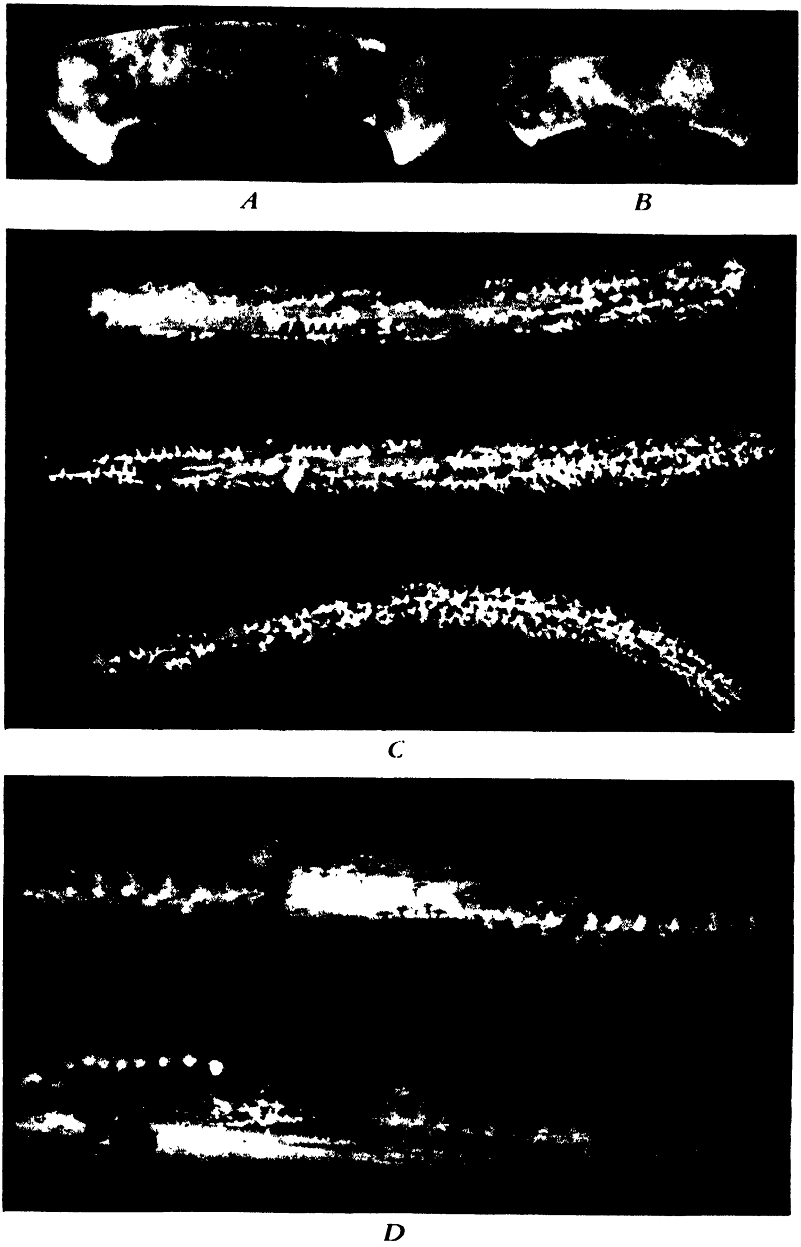

Plate 1.—*A*, Last sternite of female *Texananus lathropi* Baker, showing U-shaped indentation that extends halfway through the last ventral segment; *B*, last sternite of female *T. latipex* DeLong, showing V-shaped indentation of the posterior margin of the last ventral segment; *C*, white papillae covering the egg punctures of *T. lathropi* in the petioles of celery; *D*, papillae (enlarged) covering alternating egg punctures in two opposite rows.

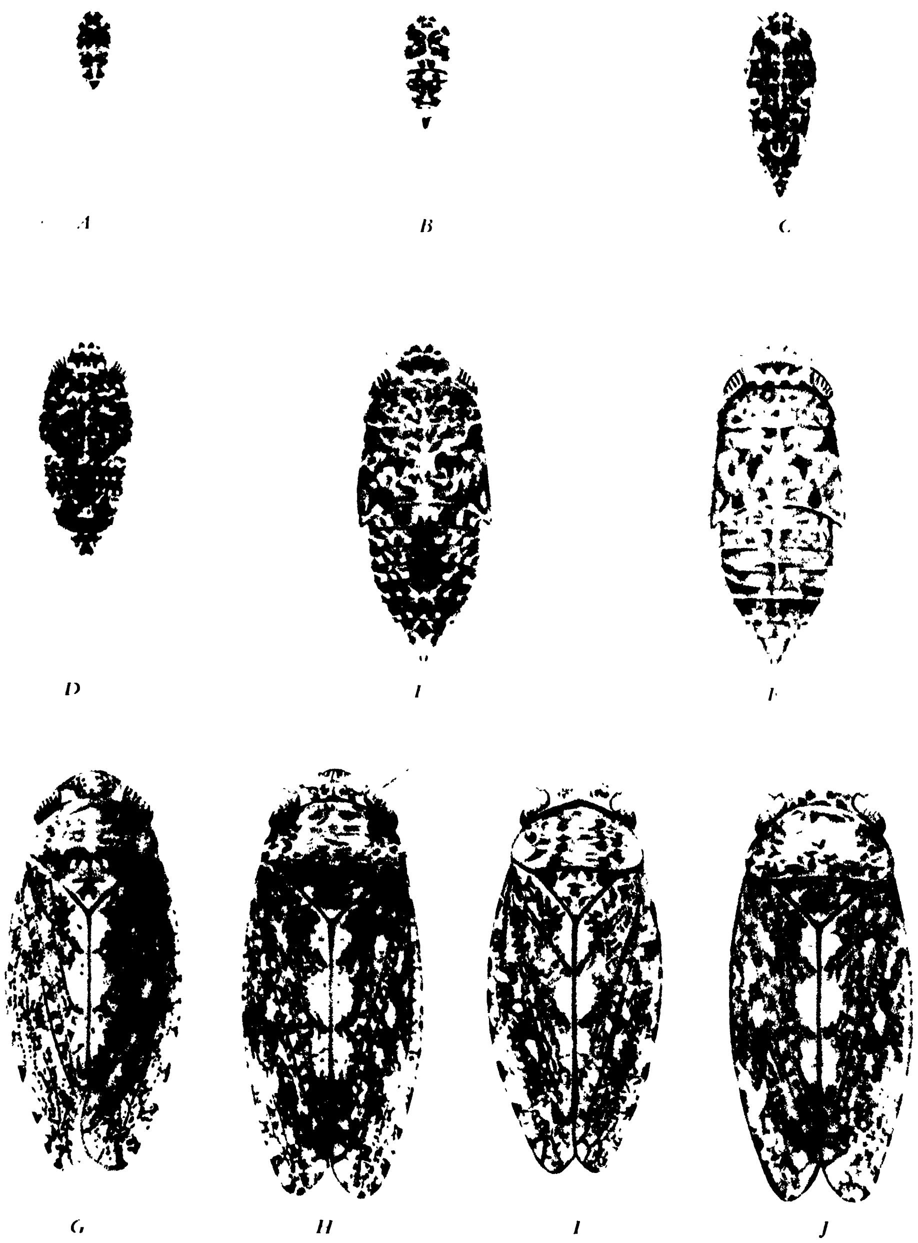

Plate 2.—Color patterns of nymphal instars and adults of *Texananus latipex* DeLong: *A*, *B*, *C*, *D*, *E*, first to fifth nymphal instars; *F*, brown fifth instar, paler than fifth instar *E*; *G*, male, *H*, female, dark forms; *I*, male, *J*, female, hyaline forms.

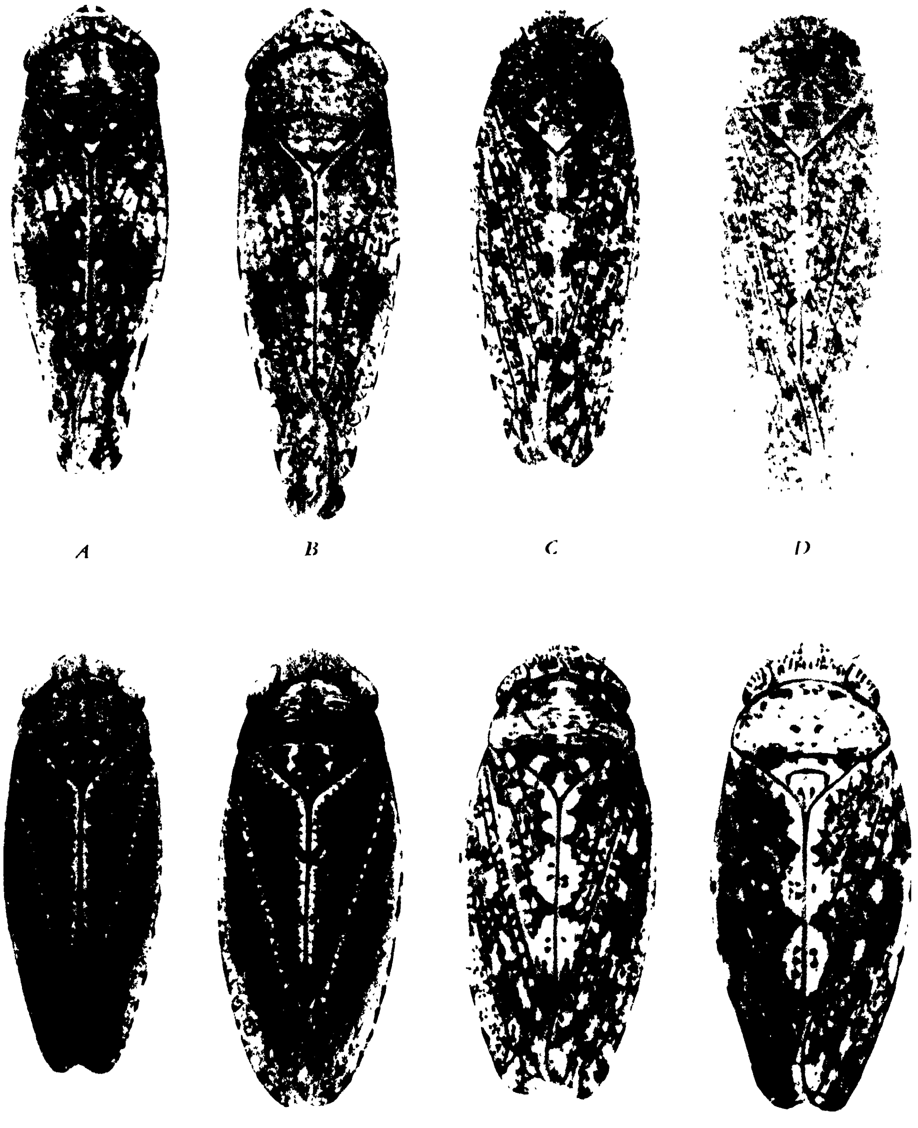

Plate 3. —*A*, Male, *B*, female ***Phlepsius apertinus*** Osborn and Lathrop; *C*, male, *D*, female ***Texananus spatulatus*** Van Duzee; *E*, male, *F*, female ***Texananus oregonus*** Ball, *G*, male, *H*, female ***Texananus pergradus*** DeLong.

of Agriculture at Salinas, California. The plants raised in the open field at Davis were transplanted at different times from Salinas. The method of reproduction in guayule was compared with that in *P. hysterophorus* L., an annual species that occurs widely in the southern states, particularly around the Gulf of Mexico, and with that in *P. incanum* H.B.K. (mariola), a perennial species.

Most of the material was collected from open-pollinated plants not subjected to any special treatment. This material, used in studying the embryo sac and embryo, is described in table 1. (In this table, lots 1–7 and 11–17 are collections from single plants; lots 8–10, mass collections.) In addition, some fertilization and controlled-pollination studies were carried out to determine the origin of the embryo and endosperm. (Only one of the plants used in these studies is listed in table 1.) The controlled-pollination studies were made with plant 3, variety 49 and plant 4, variety 427. These numbers were assigned by workers of the United States Department of Agriculture. In the text and in tables 9 and 10, the two mother plants are designated 49–3 and 427–4. In the cross-pollination experiments plant 49–3 was pollinated with pollen from a plant of Dr. W.

TABLE 1

DESCRIPTION OF MATERIAL USED IN THE STUDY

Lot no.	Approximate 2*n* number of chromosomes		Location of plant in California	Identification no.
	In plant	In accession or variety		
	Parthenium argentatum Gray (guayule)			
1	36	....	Manzanar	A6104*
2	36	......	Manzanar	A6111*
3	36	.....	Manzanar	A6113*
4	36	.....	Davis	8118-11 accession 4253†
5		36	Salinas	8118-17 accession 4253†
6		36	Salinas	8118 21 accession 4253†
7	54	.....	Salinas	8132 12 accession 4264†
8	..	54, 58, 72	Davis	Variety 111‡
9	..	72	Davis	Variety 406‡
10	..	72	Davis	Variety 593‡
11	..	72	Davis	Variety 406‡
12		72	Davis	Variety 593‡
13	72	.	Davis	Variety 49, plant 3†
14	108	..	Salinas	44 2003 45†
	Parthenium hysterophorus L.			
15	.	34	Salinas	43-699†
16	.	34	Salinas	43-700†
	Parthenium incanum H.B.K. (mariola)			
17		54	Salinas	42207-III†

* Numbers assigned by G. L. Stebbins, Jr.
† Numbers assigned by United States Department of Agriculture.
‡ Numbers assigned by W. B. McCallum.

B. McCallum's variety 406; 427–4 was crossed with a plant of his variety 444. All four plants had 72 ± as the somatic number of chromosomes.

In the fertilization studies the following combinations were used: A 36-chromosome plant of United States Department of Agriculture accession no. 4253 was pollinated with pollen from a plant of McCallum's variety 593, having 72 ± chromosome. Plant no. 593–I was pollinated with pollen from plant no. 42354–II; both of these plants had 72 ± chromosomes. The numbers of the last two plants were assigned by workers of the United States Department of Agriculture.

The emasculations and pollinations, as well as fixations of material used in the controlled-pollination and fertilization studies, were made at Salinas by Dr. R. C. Rollins.

The $2n$ chromosome numbers reported for guayule are 36, 38, 54, 58, 72, 74, and 108 to 111 (Bergner, 1944; Stebbins and Kodani, 1944). As table 1 shows, the principal chromosomal types of guayule were represented in the material used in the present study. The chromosome numbers given in the table are qualified as "approximate" because the actual numbers sometimes deviated by small amounts from those in the table. Thus, for example, the plant in lot 4 had two small chromosomes in addition to the normal 36, and the plant in lot 7 had three small chromosomes besides the 54 given in the table. Lot 8 is indicated as a mixture of individuals with different chromosome numbers. Stebbins and Kodani (1944, p. 165) published a statement that variety 111 (lot 8, table 1) "contained at least some plants with $2n = 58$." In a personal communication to the writer, Dr. G. L. Stebbins, Jr. added that $2n = 72$ and probably $2n = 54$ also occur in this variety, which appears to be a result of segregation from an unstable hybrid.

The material was fixed in chrome-acetic-formalin and in formalin-acetic-alcohol mixtures. After a fixation lasting 24 to 48 hours it was transferred directly into 70 per cent alcohol. When several changes had been made in this medium, the material was dehydrated and carried over into paraffin according to the normal butyl-alcohol schedule. The imbedding medium was a paraffin-beeswax-rubber mixture. The sections were cut 10 microns thick and were stained according to a schedule earlier described by the writer (Esau, 1944*a*).

To obtain sections of small flowers, entire heads were cut; the large flowers were removed from the heads before fixation. Usually the corolla and the upper part of the achene were cut away in the large flowers, or the ovules were dissected out.

GENERAL CHARACTERISTICS OF THE FEMALE REPRODUCTIVE STRUCTURES OF GUAYULE

Since the inflorescence and flowers of guayule were lately described and illustrated in detail by Artschwager (1943), they need not be discussed here. The development of the ovule and of the embryo sac, also considered in the literature (Kirkwood, 1910; Kokieva, 1932), must be reëxamined because the previous writers failed to distinguish between the phenomena of the sexual and asexual methods of reproduction.

A single ovule occurs at the base of the ovarian cavity (plate 1, *A*). In the initial stages of development the small protuberance that represents the primordium of the ovule is upright. The megaspore mother cell appears somewhat to one side in the hypodermal layer of the primordium. The single integument arises at the surface of the ovule, near the base of the megaspore mother cell. Since the most active cell divisions concerned with the integument formation occur above the laterally placed megaspore mother cell, the ovule becomes inverted, and the micropyle eventually points downward in the ovarian cavity (plates 1 and 5).

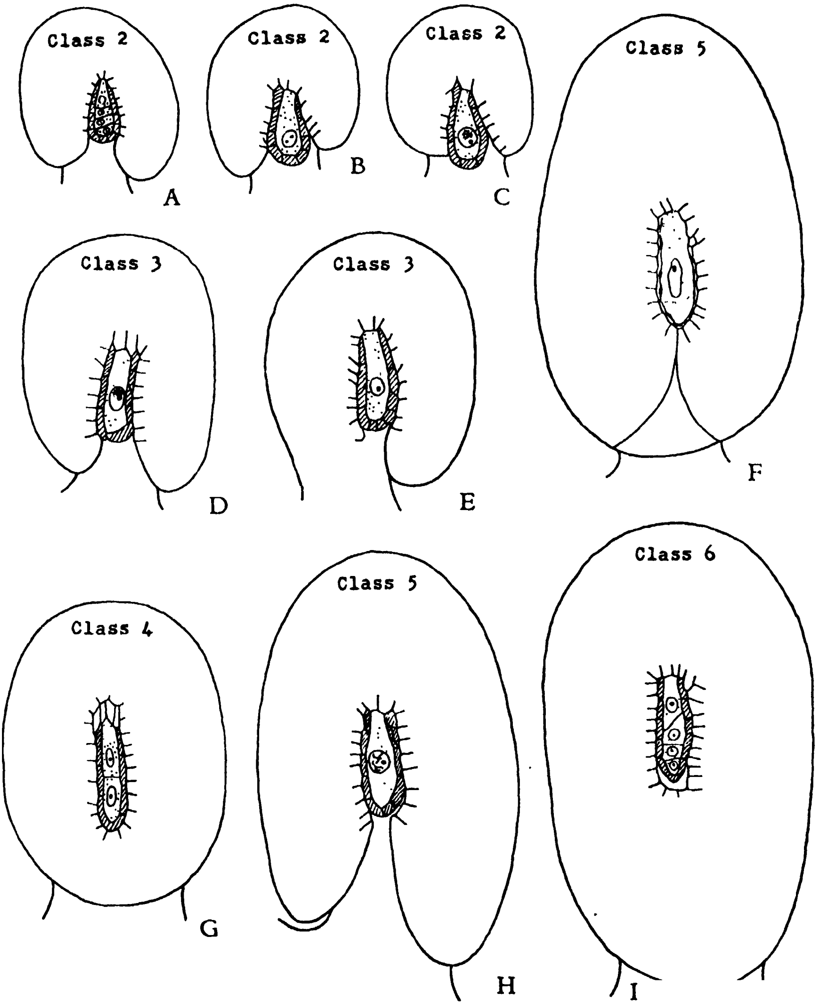

Fig. 1.—Diagrams of longitudinal sections of guayule ovules containing megaspore mother cells in resting stage (*B, E,* and *F*) and in meiotic prophases (*C, D,* and *H*); a dyad (*G*); and tetrads of megaspores (*A* and *I*). The ovule in *A* is from a 36-chromosome plant; the others are from a 72-chromosome type. In all drawings the protoplasts of the megaspore mother cells and of the dyad and tetrad members are stippled. The nucellar epidermis enclosing the generative cells is singly hatched. The nucellus projects beyond the integument in *B* and *C*; in all other figures it is partially or completely enclosed by the integument. The funiculus is indicated at the base of each ovule. The size classes of the ovules are given on the drawings. (All × 194.)

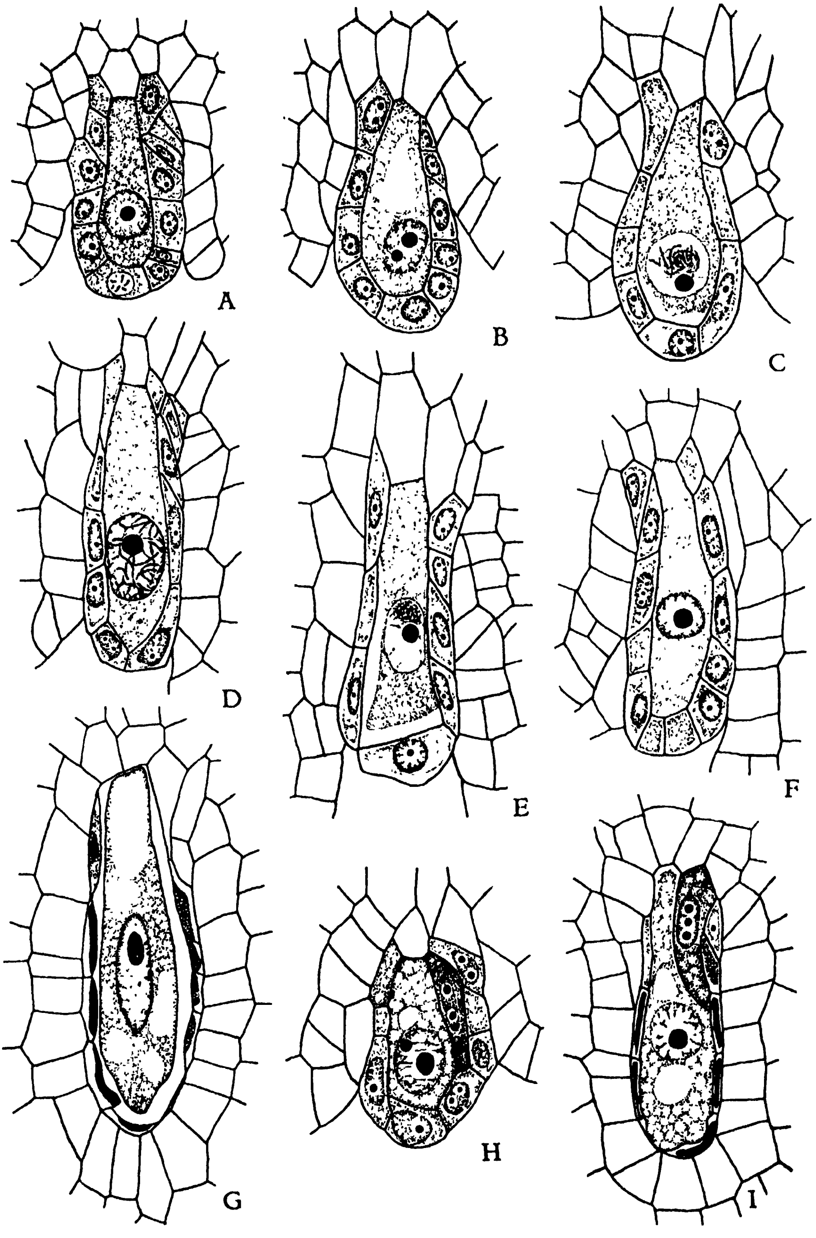

Fig. 2.—Longitudinal views of megaspore mother cells and enclosing nucellar epidermal cells from 72-chromosome guayule. The protoplasts of the megaspore mother cells are stippled; those of the nucellar epidermis are stippled or are shown in black. The blackening indicates that the cells are partly crushed. In *A–G* one megaspore mother cell occurs in each nucellus; in *H* and *I* two such cells, a large one and a small one, are present in the same nucellus. The megaspore mother cells in *C, D,* and *E* are in meiotic prophases; all others are resting. The drawings were made from ovules of the following size classes (see fig. 1 and text): *A–C* and *H,* class 2; *D–F,* class 3; *I,* class 4; *G,* class 5. (All × 600.)

In keeping with the original terminology (Schneider, 1917), the ovule is here interpreted as composed of the nucellus (which constitutes the inner portion of the ovule), the integument, and the funiculus. No sharp line of demarcation can be drawn between any two of these three parts. The megaspore mother cell occurs at one end of the nucellus, and most of it is covered only by the nucellar epidermis.[6] The latter forms a distinct uniseriate layer that grows by anticlinal divisions and tangential stretching of cells during the enlargement of the megaspore mother cell (figs. 1 and 2; plates 1, *A* and *B;* 2; 3, A).

In the normal cycle of reproduction, the megaspore mother cell passes through the usual stages of meiosis (plate 2, *A* and *B;* plate 3, *A* and *B*). The first meiotic division is followed by cytokinesis (plate 2, *C*), and dyads are formed (plate 2, *D*). The dyad nuclei typically occur in the stage of interkinesis (plate 2, *C* and *D*). The second meiotic division gives rise to tetrads of megaspores. These are arranged in one longitudinal row (plate 2, *E* and *F*); or the two megaspores nearest the micropyle occur laterally side by side, and then certain longitudinal sections show only three of the tetrad members (plate 3, *C*).

The cytoplasm of the megaspore mother cell, of the dyads, and of the young tetrads is relatively uniform throughout the cell, with no conspicuous vacuoles (plate 2). The chalazal megaspore, which becomes the embryo sac, is, from the beginning, the largest member of the tetrad (plate 2, *E*). Upon starting to elongate, it develops a prominent, often sharply delimited vacuole at the chalazal end (plate 3, *C*). The three megaspores toward the micropylar end are gradually crushed by the functioning megaspore. The cell nearest to the latter usually is destroyed first (plate 3, *C*). When the functioning megaspore reaches the stage of a uninucleate embryo sac, it commonly develops one or more large vacuoles in its micropylar end. The nucleus then becomes located in the relatively dense central part of the young sac, with highly vacuolated areas on either side of it (plate 3, *D*). Fully developed uninucleate embryo sacs associated with completely crushed megaspores (as in plate 3, *D*) are rarely encountered in sections. Apparently the enlargement of the functioning megaspore is quickly followed by the nuclear division that forms the binucleate embryo sac. The two nuclei first occur in the center of the embryo sac (plate 3, *F*), then migrate to the two opposite ends of it (plate 3, *E*).

The nucellar epidermis is stretched and crushed during the development of the uninucleate and binucleate embryo sacs. Commonly this process begins at the sides of the tetrad group or of the young embryo sac (plate 3, *C–F*). Eventually the nucellar epidermis is pierced, either at the side of the embryo sac or at its micropylar end; and the embryo sac emerges into the cavity formed by the overgrowing integument (plate 4, *B* and *C*). The remnants of the crushed megaspores usually are evident as long as the embryo sac has one or two nuclei (plate 3, *D–F*). After the nucellus is pierced, these remnants become indistinguishable, though sometimes they may be detected at the tetranucleate stage on one or the other side of the embryo sac.

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[6] Satina (1945) has suggested denoting as *nucellus* only the part of the ovule that originates in the subepidermal layer of the placenta, and distinguishing it from the derivatives of the outer layer of the placental wall. According to this definition, the megaspore mother cell is partly imbedded in the nucellus, but on the periphery it is covered by nonnucellar cells. One might question the usefulness of such a partitioning of an organ based on the origin of parts in the meristem: the fate of the histogen theory has shown the fallibility of the "attempt to assign specific destinies... to the various regions of the meristem" (Foster, 1939, p. 457). The only reason for retaining the term *nucellus* in modern literature seems to be the convenience in describing ovules; for the sake of simplicity, it may well continue to be used with reference to the main body of the ovule within the integuments, including the epidermis that covers the sporogenous tissue.

After the nucellar epidermis is destroyed, the embryo sac comes into contact with the inner epidermis of the integument. During the dyad stage this epidermis begins to assume its characteristic appearance—that of a layer of rather densely staining, orderly arranged cells lining the cavity that was formed by the integument back of the micropyle (plates 2, *D*; 3, *C–F*; 5, *A*). With further enlargement of the integumentary cavity, the cells lining it divide anticlinally and become narrow, with their long diameters perpendicular to the periphery of the cavity (plates 1, *C*; 4, *B* and *C*). In keeping with the common terminology, this layer is here called the "nutritive jacket," though its real function is still obscure. Possibly some nucellar cells also take part in forming the nutritive jacket, since in later stages of development this tissue almost encloses the embryo sac (figs. 4 and 5; plates 11–13).

In the apomictic cycle the nuclear division in the megaspore mother cell is delayed, but the latter enlarges and vacuolates, assuming the appearance of a uninucleate embryo sac. Figure 2, *A*, *C–E*, plates 2, *A* and *B*, and 3, *A*, show megaspore mother cells with normal-appearing cytoplasm free of large vacuoles. Several of these cells are in meiotic prophases (fig. 2, *C–E*; plates 2, *A* and *B*; and 3, *A*). In contrast, figure 2, *B*, *F*, and *G*, and plate 4, *A*, illustrate megaspore mother cells with conspicuous vacuoles in their cytoplasm. Such vacuolating megaspore mother cells are common in material taken from apomictic plants, while those with the relatively dense cytoplasm are characteristic of the sexually reproducing guayule.

Before its nucleus divides, the enlarging megaspore mother cell of the apomictic guayule frequently crushes the nucellar epidermis or even pierces it. In figure 2, *G* and *I*, and plate 4, *A*, vacuolated megaspore mother cells are associated with a more or less crushed nucellar epidermis. The absence of crushed megaspores in these views proves that these cells are not the reduced uninucleate embryo sacs formed after meiosis. Such megaspore mother cells may be conveniently called unreduced, or aposporic, or apomeiotic embryo sacs, because upon division they give rise to binucleate structures, whose further development resembles that of ordinary binucleate embryo sacs.

As was mentioned previously, views of divisions are rarely encountered in the megaspore mother cells of the apomicts. Those observed were not sufficient to show whether a semiheterotypic or a purely mitotic division occurs in the formation of the binucleate embryo sac from the unreduced uninucleate structure. Immediately after the first division, the unreduced binucleate embryo sac differs strikingly from the dyads. In the latter the nuclei are usually in interkinesis, with the chromatic units sharply outlined on their periphery (plate 2, *C* and *D*); and the cell plate appears as a narrow line between the two nuclei (plate 2, *C*). Just after formation the unreduced binucleate embryo sac has two nuclei, with the chromatin more or less netted in appearance and spread out through the nucleus. Instead of a cell plate between the nuclei, there is a wide band of somewhat darkly stained material (probably remnants of the spindle fibers), which later disappears (plate 6, *B*). Belated meiotic divisions and formation of tetrads occurred in much-enlarged megaspore mother cells, which were contained in ovules considerably larger than those usually associated with the stage of meiosis.

The degree of predivisional enlargement of the aposporic uninucleate embryo sac varies. The unreduced binucleate structure in plate 6, *B*, is still enclosed within the nucellus. In plate 6, *A*, an enlarged megaspore mother cell has pierced the nucellar epidermis; although the remnants of the latter are very conspicuous, no megaspores are present. Sometimes the young aposporic embryo sac grows so vigorously that it pushes through the micropyle into the ovarian cavity; its nuclei then lie free in this cavity, surrounded only by the cytoplasm of the embryo sac (plate 6, *C*). (In some of the older ovules, embryos occurred in the micropyle and in the ovarian cavity. They probably developed in embryo sacs that protruded beyond the usual confines.)

After the binucleate stage is reached, the development of the embryo sac is similar in the normal and aposporic cycles of reproduction. The two nuclei of the binucleate embryo sac divide to form a tetranucleate embryo sac (plate 4, *B* and *C*). Each of the four nuclei divides again, giving rise to the eight-nucleate embryo sac. Since this last nuclear division in the maturation of the embryo sac is immediately followed by cytokinesis, cell plates appear between the members of each of the four pairs of new nuclei. Plate 7, *A* and *B*, illustrates this stage of development. In this plate, nuclei 1–3 are antipodal nuclei; 4 and 5, polar nuclei; 6, the egg nucleus; 7 and 8, synergid nuclei. No cell plate was yet evident between the nuclei 2 and 3, but one was about to be formed. Perhaps this wall is sometimes omitted, for guayule embryo sacs may show two, sometimes three antipodals (figs. 4; 5; 6, *A* and *B;* plate 7, *D*).

In older embryo sacs, the antipodal nuclei are rather large and foamy, with more than one nucleolus (fig. 6, *A* and *B*). The antipodals may have more than one nucleus, or the single large nucleus is lobed. Often the antipodals persist long after the embryo starts developing. The two synergids show the usual vacuolation of the broad end turned toward the interior of the embryo sac (plate 8, *A*). The dense narrow end pointing toward the micropyle contains the nuclei. In the preparation of the material for microscopic study, the vacuolated part of the synergids commonly collapses, and the synergids assume the peculiar shape illustrated in plate 8, *B*. The polars fuse soon after being formed (plate 8, *B*), and the polar fusion nucleus occurs near the egg in the mature embryo sac (plate 9, *B*). The egg is of the usual type, with a vacuole in the narrow end near the micropyle and a nucleus in the broad dense end turned toward the interior of the sac. The eggs of the 36-chromosome guayule are obviously smaller than those of the predominantly apomictic 72-chromosome plants (plate 9). The polar fusion nuclei also differ in the two kinds of plants. Those in the 36-chromosome plants are rounded or somewhat elongated structures, each with one large nucleolus (plate 9, *B* and *C*); in the apomictic plants the polar fusion nucleus commonly becomes large and lobed and contains many nucleoli (fig. 6, *H–J;* plates 8, *C;* and 12, *C*). When all parts of the embryo sac are differentiated, seven cells are present in it: the egg, the two synergids, the three antipodals, and the large central primary endosperm cell that contains the polar fusion nucleus. If there are only two antipodals, the embryo sac is divided into six cells only. For convenience, however, the mature embryo sac is here always called the "7-celled embryo sac."

The abnormalities in the embryo and endosperm development will be considered elsewhere in this paper. The apparently normal development for guayule may, however, be outlined briefly. Plate 11, *A* and *B*, shows two very young embryos. Both are associated with a developing endosperm, which, from the beginning, is usually cellular. A somewhat older embryo and a cellular endosperm are depicted in plate 11, *C*. The embryo in plate 12, *D* is at the stage just before the cotyledons emerge. The latter had been initiated in the embryo in plate 13, *A*. In plate 13, *B*, the embryo has prominent cotyledonary primordia and a procambial system; and in 13, *C*, it is mature. In the young achenes the cotyledons are oriented with their broad sides perpendicular to the broad sides of the achene (fig. 5, *I* and *L;* plate 13, *B*), whereas in the mature state the broad sides of the cotyledons usually parallel the broad sides of the achene (plate 13, *C*). Seemingly, at some time between the stages depicted in plates 13, *B* and *C*, the embryo turns about halfway around its longitudinal axis.

During the development of the embryo, the sac containing it also enlarges. The nutritive jacket, which remains uniseriate, is much stretched. The ovular tissues (parts of the nucellus and of the integument) are gradually destroyed and, presumably, absorbed. Plate 5, *C*, illustrates the dense staining of the ovular cells that are the first to break down during the enlargement of the sac. The collapse of these cells is initiated on the flanks of the embryo sac (plates 11 and 12); later (plate 13, *A* and *B*) it is more prominent at the chalazal end. (The breaks at the top of the ovules in plate 13, *A* and *B*, were caused by the removal of corollas in preparation for fixation; the collapse of cells near the embryo sac is natural.) No special cells connect the vascular tissue with the embryo sac. The single vascular bundle passes near the periphery of the ovule from the funiculus into the outer part of the integument and ends blindly without reaching the tip of the ovule (plate 5, *C*).

COMPARISON OF EMBRYO-SAC FORMATION IN THE DIFFERENT CHROMOSOMAL TYPES OF GUAYULE AND IN MARIOLA

Two features—the abundance of enlarging, vacuolating, but resting megaspore mother cells and the scarcity of megaspores—proved to be the best criteria for recognition of apomeiosis in this study. In an attempt to estimate the duration of the different stages of embryo-sac development, these stages were related to the sizes of the ovules in which they occurred. The ovules were measured between the point of attachment of the funiculus to the ovary wall and the top of the ovule. For the preparation of tables 2, 3, 5, and 6 the ovules were sorted into size classes at 50-micron intervals. The first class contained the smallest ovules, 50 to 100 microns high; the eighth class the largest ovules, 400 to 450 microns high. (Ovules of size 8 are not given in table 2, since those belonging to this class in the 36-chromosome plants contained only 7-celled and older embryo sacs. They were relatively older than the ovules of class 8 in the polyploid plants.)

In the tables, the term "resting megaspore mother cells" is applied to those whose nuclei were not preparing for division. Figure 2, *A*, *B*, *F*, and *G*, and plates 4, *A*, and 6, *A*, illustrate such megaspores. The various stages of prophase (fig. 2, *C–E;* plates 2, *A* and *B,* and 3, *A* and *B*), as well as the metaphases and anaphases, identified the "dividing megaspore mother cells." The dyads are, as a rule, clearly defined; and they are distinct from the tetrads of megaspores. Since, however, the crushing of the nonfunctioning megaspores is a gradual process, the line of division between the tetrad stage and that of the reduced uninucleate embryo sac must be drawn somewhat arbitrarily. When the nonfunctioning megaspores occupied a fraction of their original space (but were not necessarily entirely crushed), the stage was called "uninucleate embryo sac with crushed megaspores." For identifying the occurrence of meiosis in the reproductive cycle, the reduced uninucleate embryo sacs are as useful as the tetrads of megaspores. The remnants of the crushed megaspores are, as a rule, easily detected in the uninucleate-embryo sac stage; their final obliteration occurs in the older stages. In the tables the presence of remnants of megaspores in stages beyond that of the uninucleate embryo sac was disregarded, because their absence in these stages did not necessarily indicate that they were not present at any time: they may have been crushed beyond recognition. The binucleate, tetranucleate, and 7-celled embryo sacs are easily distinguished from one another. If the contents of the ovule were too much disorganized for identification, they were called "degenerated material."

TABLE 2

RELATION BETWEEN SIZE OF OVULE AND STAGE OF EMBRYO-SAC DEVELOPMENT IN 36-CHROMOSOME GUAYULE, AS DETERMINED BY CLASSIFYING 346 OVULES OF LOT 4 IN TABLE 1

Contents of ovules	Percentages of different contents in ovules of the various size classes							Total per cent for each item
	1	2	3	4	5	6	7	
Resting megaspore mother cells	2.6	....	....	....	....	....	....	2.6
Dividing megaspore mother cells	10.1	17.1	2.3	....	....	....	....	29.5
Dyads	....	1.4	0.9	0.6	....	....	....	2.9
Tetrads of megaspores	...	2.6	8.4	4.9	....	....	....	15.9
Uninucleate embryo sacs with crushed megaspores	...	0.9	0.9	7.5	1.7	....	....	11.0
Binucleate embryo sacs	....	0.3	1.4	6.1	5.9	1.3	....	15.0
Tetranucleate embryo sacs	....	....	....	2.6	3.3	1.9	0.8	8.6
Seven-celled embryo sacs	....	....	....	1.8	3.4	3.8	5.5	14.5
Total	12.7	22.3	13.9	23.5	14.3	7.0	6.3	....

Table 2 relates the stage of embryo-sac development to the size of the ovule in 36-chromosome guayule. With the increase in ovule size, the number of the megaspore mother cells quickly falls off, because most of these cells undergo meiosis and become dyads and tetrads. The dyad stage is apparently brief, as evidenced by the small percentage of ovules showing dyads. With further growth of ovules, the tetrad stage is followed by the successive stages of embryo-sac development. The orderly succession of stages with the increase in ovule size is clearly demonstrated by the downward displacement of the figures from left to right in table 2. The different stages did not often overlap, except that the binucleate embryo sacs were encountered in five different sizes of ovules. The last column ("total per cent for each item") emphasizes two features. A great majority of the megaspore mother cells were in division; and megaspores, intact or more or less crushed (items "tetrads of megaspores" and "uninucleate embryo sacs with crushed megaspores"), were common in sections. Degenerative stages were not encountered in this group of ovules.

The relation between ovule size and stage of embryo-sac development in 72-chromosome guayule strikingly contrasts with that in the 36-chromosome plants (table 3). Among all types of structures the resting megaspore mother cells are the most abundant, and they occur in all the size classes of ovules. Figure 1 illustrates the sizes of ovules containing megaspore mother cells and their products. The ovule in figure 1, *A*—one from a 36-chromosome plant—contains tetrads. It is an ovule of the smallest size class that shows tetrads in table 2. Figure 1, *B–I*, drawn from ovules of 72-chromosome guayule, illustrates the lack of orderly relation between ovule size and the stage of development of the contents. Megaspore mother cells are shown in ovules of classes 2, 3, and 5 (fig. 1, *B–F*, and *H*); the ovule of class 4 in figure 1, *G*, contains dyads, while the larger ovules in figure 1, *F* and *H*, still contain megaspore mother cells. The meiotic prophase in the ovule of class 5 (fig. 1, *H*) and the tetrads in the ovule of class 6 (fig. 1, *I*) indicate that meiosis may occur relatively late in the development of the ovule. Plate 5, *A* and *B*, compares for size (at different magnifications) an ovule of class 5 from a 36-chromosome plant containing a dyad and another of class 8 from a 72-chromosome plant containing a megaspore mother cell that had crushed the nucellar epidermis (unreduced uninucleate embryo sac).

TABLE 3

Relation between Size of Ovule and Stage of Embryo-Sac Development in 72-Chromosome Guayule, as Determined by Classifying 953 Ovules of Lots 11 and 12 in Table 1

Contents of ovules	Percentages of different contents in ovules of the various size classes								Total per cent for each item
	1	2	3	4	5	6	7	8	
Resting megaspore mother cells	4.2	10.8	16.6	14.7	9.4	4.6	2.5	0.1	62.9
Dividing megaspore mother cells	....	0.4	2.3	3.5	1.2	0.3	0.2	0.1	8.0
Dyads	....	....	0.1	0.6	0.2	0.2	....	....	1.1
Tetrads of megaspores	....	....	0.1	1.6	2.5	2.2	0.8	0.3	7.5
Uninucleate embryo sacs with crushed megaspores	....	....	....	0.2	1.0	0.3	0.2	....	1.7
Binucleate embryo sacs	....	....	0.2	2.1	2.6	2.6	3.1	1.5	12.1
Tetranucleate embryo sacs	....	....	0.3	0.1	1.2	0.7	1.6	0.4	4.3
Seven-celled embryo sacs	....	....	....	....	....	....	0.7	0.1	0.8
Degenerated material	....	....	0.1	0.6	0.2	0.1	0.5	0.1	1.6
Total	4.2	11.2	19.7	23.4	18.3	11.0	9.6	2.6	....

As was stated previously, the megaspore mother cell persists through many developmental stages of ovules in 72-chromosome guayule (table 3), because the division that gives rise to an unreduced binucleate embryo sac is delayed. Some of the resting megaspore mother cells listed in table 3 had reached the stage of unreduced uninucleate embryo sac (plate 4, *A*). Dividing megaspore mother cells were encountered rarely, and most of these were in meiotic prophases (fig. 2, *C–E;* plate 3, *A* and *B*). The scarcity of dyads shown in table 3 is not a criterion for omission of meiosis, since dyads are rare also in the material from normally reproducing plants (table 2). The difference in percentages of views with megaspores ("tetrads of megaspores" and "uninucleate embryo sacs with crushed megaspores") in tables 2 and 3 is significant: the small numbers of such views in the 72-chromosome material (table 3) is good evidence of frequent omission of meiosis. Their presence, on the other hand, proves that apomeiosis is not total in the 72-chromosome plants, but that it is combined with production of spores. Table 3, in contrast to table 2, indicates no orderly sequence of events in the development of the embryo sacs: the figures are spread out all over the table, instead of dropping down from left to right with the increase in ovule size. Indeed, various young stages occurred in ovules still larger than those recorded in table 3. Ultimately many of these young embryo sacs cease developing and become disorganized. Even in the young ovules used for table 3, the contents had sometimes degenerated (item "degenerated material").

In studying the nature of reproduction, one should always use plants that have developed under favorable environmental conditions. A situation that affects adversely the development of flowers may obscure the differences between the normal and apomeiotic plants. The 36-chromosome plant of lot 4 (tables 1 and 2) was for a time affected by thrips, and the flowers were making little progress in development. The 65 ovules collected at this time showed 29.2 per cent of resting and 16.9 per cent of dividing megaspore mother cells; no dyads; 4.6 per cent of tetrads and uninucleate embryo sacs with crushed megaspores; and 30.8 per cent of degenerated structures. The remaining 18.5 per cent of ovules contained embryo sacs older than the uninucleate. (These figures were not used in preparing table 2.) The high per cent of resting megaspore mother cells apparently was not associated with apomeiosis, because all these cells occurred in small ovules (23.1 per cent in size 1; 4.6 per cent in size 2; 1.5 per cent in size 3). They seemed to have stopped developing and eventually degenerated. Nevertheless, this material could have been confused with the apomictic if other samplings from the same plant had not yielded entirely different results (table 2).

As was previously shown (Esau, 1944*b*), the degree of megasporogenesis or apomeiosis may be estimated from data like those in tables 2 and 3. The item "resting megaspore mother cells" may be considered as indicating apospory, the four items "dividing megaspore mother cells," "dyads," "tetrads of megaspores," and "uninucleate embryo sacs with crushed megaspores" may be looked upon as evidences of megasporogenesis. Naturally, this estimate is an approximation: not all the megaspore mother cells that were resting at the time of sampling would have omitted meiosis; but, on the other hand, the division of the nucleus in the dividing megaspore mother cells was not necessarily meiotic.

Table 4 compares megasporogenesis in different lots of 72-chromosome guayule and estimates the degree of apomeiosis in these lots. The ovules that contained binucleate, tetranucleate, and 7-celled embryo sacs were excluded from the calculations for table 4. To emphasize that many of the resting megaspore mother cells differentiated into unreduced uninucleate embryo sacs, the enlarged megaspore mother cells associated with crushed nucellar epidermis were counted separately under "uninucleate embryo sacs with crushed nucellar epidermis" (table 4). The values for resting megaspore mother cells and uninucleate embryo sacs with crushed nucellar epidermis were added in an attempt to estimate the per cent of apomeiosis (table 4, last column). The difference between this total and 100 per cent, that is, the total of the fifth, sixth, seventh, and ninth columns, would represent the per cent of flowers showing megaspore formation. The range of variation of per cent of apomeiosis (from 69.0 per cent to 90.9 per cent) in the different samples and the average of 77.8 per cent compare favorably with the findings on apomixis by Powers and Rollins (1945). In the work of these authors the degree of apomixis was revealed by the percentage of maternal types of plants in the F_1 generation from interspecific crosses. In the F_1 progenies of 16 plants of 72-chromosome guayule crossed with mariola, the percentage of maternals varied from 61.6 ± 6.8 to 95.2 ± 4.7, with an average of 85.58 ± 1.2 (Powers and Rollins, 1945, table 4, p. 107).

TABLE 4

MEGASPOROGENESIS IN 72-CHROMOSOME GUAYULE

Lot no. in table 1	Date of collection	Number of ovules examined	Percentages of ovules showing generative tissue in the following stages						Estimated per cent of apomeiosis†
			Resting megaspore mother cells	Dividing megaspore mother cells	Dyads	Tetrads	Uninucleate embryo sacs with crushed nucellar epidermis*	Uninucleate embryo sacs with crushed megaspores	
9	April, 1943........	341	65.1	14.1	2.1	8.2	9.1	1.4	74.2
10	April, 1943.........	88	56.8	14.8	0.0	13.6	14.8	0.0	71.6
11	May 2, 1944.......	242	62.8	15.7	2.1	7.4	11.6	0.4	74.4
11	August 21, 1944....	162	56.2	6.2	1.2	12.9	17.3	6.2	73.5
12	May 2, 1944........	208	67.3	6.7	1.0	1.4	23.6	0.0	90.9
12	August 21, 1944....	161	52.8	8.7	1.2	18.0	16.2	3.1	69.0
13	June and July, 1944	184	83.7	7.6	0.0	3.8	4.9	0 0	88.6
	Average.........	....	*64.5*	*10.9*	*1.3*	*8.5*	*13.3*	*1.6*	*77.8*

* Enlarged megaspore mother cells.

† Sum of per cent of resting megaspore mother cells and of uninucleate embryo sacs with crushed nucellar epidermis.

In table 4, lots 11–13 were derived from single plants; lots 9 and 10 from groups of plants. The collections of lots 11 and 12 were made at two different dates (table 4). In lot 11 the two collections gave comparable values for per cent of apomeiosis. In lot 12, however, the May sample showed 90.9 per cent of apomeiosis, as contrasted with 69.0 in the August collection. The chief difference between these two collections in lot 12 is in the percentage of views showing tetrads and crushed megaspores—views that are not easily misinterpreted. Externally there was no evidence of any kind of injury to the plant at one or the other time during the season. This instance of variability in degree of apospory in samples taken at different times permits no conclusion regarding seasonal influences upon the method of reproduction, but it suggests that such influence may occur.

In both lots 11 and 12, the two collections differed in the vigor of growth of the aposporic embryo sacs; sacs that had emerged into the ovarian cavity occurred mostly in the August collection (plate 6, *C*).

Lots 11–13, being single-plant collections, show that meiosis and apomeiosis occur in the same plant (facultative apomeiosis). Views indicating megaspore formation and apomeiosis occur among flowers of the same head. An attempt was made to determine whether there was any tendency toward dominance of one or the other kind of flower in a given head. Figure 3 shows the relative frequency of flower heads with the different numbers of normal flowers. Apparently the normal flowers are distributed perfectly at random among the aposporic ones.

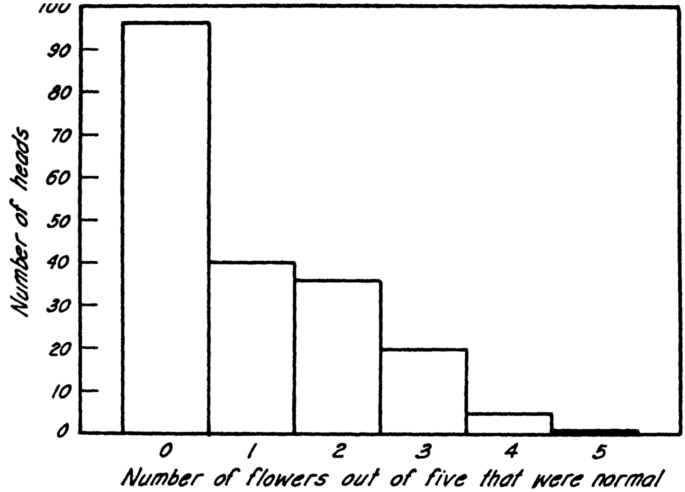


Fig. 3.—Diagram showing the relative frequency of flower heads with the different proportions of normal flowers. This diagram was based on a study of 195 heads of 72-chromosome guayule.

The degree of apomeiosis in the 36-chromosome material was estimated as 4.2 per cent (table 7). Even this value may be too high, because, for example, the 2.6 per cent of resting megaspore mother cells in table 2 occurred in the youngest ovules and probably would have undergone meiosis later on. The 0.3 per cent of uninucleate embryo sacs with crushed nucellar epidermis in table 7, however, is a good indication of apomeiosis. The embryo that was found in an achene from a self-pollinated, self-sterile 36-chromosome plant also suggests apomictic reproduction, though 36-chromosome plants are not always entirely self-incompatible. Powers and Rollins (1945, table 5, p. 110) obtained 3.8 per cent of seedlings upon selfing one of the 36-chromosome plants. On the other hand, their data also indicate that apomeiosis may occur in these plants. In the F_1 generation from a cross between a 36-chromosome guayule and mariola, no maternals occurred (Powers and Rollins, 1945, table 4, p. 107); but among the hybrids 3.6 per cent were of aberrant type. According to a personal communication from Dr. R. C. Rollins, the aberrants were presumably triploids that arose through fertilization of unreduced, 36-chromosome eggs. The 3.6 per cent of nonreduction agrees well with the 4.2 per cent apomeiosis estimated for the 36-chromosome material in the present study.

TABLE 5

RELATION BETWEEN SIZE OF OVULE AND STAGE OF EMBRYO-SAC DEVELOPMENT IN 54-CHROMOSOME GUAYULE, AS DETERMINED BY CLASSIFYING 285 OVULES OF LOT 7 IN TABLE 1

Contents of ovules	Percentages of different contents in ovules of the various size classes								Total per cent for each item
	1	2	3	4	5	6	7	8	
Resting megaspore mother cells...	3.5	10.2	9.1	10.6	6.9	2.5	1.8	....	44.6
Dividing megaspore mother cells.	....	....	2.8	0.7	0.9	0.5	...	...	4.9
Dyads.........................	....	....	....	....	0.2	0.2	....	...	0.4
Tetrads of megaspores...........	....	....	....	....	0.4	0.3	....	....	0.7
Uninucleate embryo sacs with crushed megaspores...........	....	....	....	....	....	....	....	....	....
Binucleate embryo sacs..........	....	....	0.3	0.7	4.4	3.3	4.9	1.1	14.7
Tetranucleate embryo sacs.	....	....	....	0.3	2.0	2.6	7.0	2.1	14.0
Seven-celled embryo sacs....	....	....	....	...	....	....	1.8	0.7	2.5
Degenerated material....	....	0.3	....	1.1	5.9	7.0	3.2	0.7	18.2
Total..	3.5	10.5	12.2	13.4	20.7	16.4	18.7	4.6	....

TABLE 6

RELATION BETWEEN SIZE OF OVULE AND STAGE OF EMBRYO-SAC DEVELOPMENT IN 54-CHROMOSOME MARIOLA, AS DETERMINED BY CLASSIFYING 235 OVULES OF LOT 17 IN TABLE 1

Contents of ovules	Percentages of different contents in ovules of the various size classes								Total per cent for each item
	1	2	3	4	5	6	7	8	
Resting megaspore mother cells..	11.9	7.2	15.3	11.0	4.3	0.4	....	0.4	50.5
Dividing megaspore mother cells.	....	3.4	0.9	1.3	....	....	....	....	5.6
Dyads.........................	....	....	....	....	....	....	....	....	....
Tetrads of megaspores.....	....	....	....	....	....	....	....	...	....
Uninucleate embryo sacs with crushed megaspores...........	....	....	....	....	....	....	....	. ..	...
Binucleate embryo sacs..........	....	0.4	....	1.7	1.4	1.2	0.9	0.4	6.0
Tetranucleate embryo sacs.......	...	....	....	0.4	1.0	0.3	....	0.4	2.1
Seven-celled embryo sacs.........	.	....	....	....	2.8	2.7	2.6	1.7	9.8
Degenerated material............	1.3	0.9	4.7	8.5	5.0	3.1	2.5	....	26.0
Total........................	13.2	11.9	20.9	22.9	14.5	7.7	6.0	2.9	....

Table 5 relates embryo-sac development to ovule size in a 54-chromosome guayule plant. The course of events essentially resembles that in the 72-chromosome material. Here, too, the predominance of resting megaspore mother cells, their persistence during ovule enlargement, and the scarcity of views with megaspores point to facultative apomeiosis. Degeneration of contents of ovules was more prominent in this plant than in the 72-chromosome lots. The estimated per cent of apomeiosis was 88.2 (table 7, lot 7). A mass collection from the mixed polyploid variety marked lot 8 in table 1, which contained some 54- and 58-chromosome individuals, showed only 52.1 per cent of apomeiosis (table 7, lot 8).

A 108-chromosome type of plant (table 1, lot 14) exhibited a rather low percentage of apospory. Its estimated per cent of apomeiosis was 56.0 (table 7). The resting megaspore mother cells occurred in ovules of classes 1–7; and among 164 ovules only 3.7 per cent contained degenerated material.

TABLE 7

MEGASPOROGENESIS IN GUAYULE WITH VARIOUS NUMBERS OF CHROMOSOMES, AND IN MARIOLA

Lot no. in table 1	Approximate 2*n* number of chromosomes	Number of ovules examined	Percentages of ovules showing generative tissue in the following stages						Estimated per cent of apomeiosis†
			Resting megaspore mother cells	Dividing megaspore mother cells	Dyads	Tetrads	Uninucleate embryo sacs with crushed nucellar epidermis*	Uninucleate embryo sacs with crushed megaspores	
				Guayule					
1–6	36	311	3.9	40.5	4.8	31.5	0.3	19.0	4.2
7	54	144	69.4	9.7	0.7	1.4	18.8	0.0	88.2
8	54, 58, 72	119	43.7	46.2	0.0	1.7	8.4	0.0	52.1
9-13	72	1,386	64.5	10.9	1.3	8.5	13.3	1.5	77.8
14	108	116	49.1	27.6	1.7	13.8	6.9	0.9	56.0
				Mariola					
17	54	132	75.0	9.8	0.0	0.0	15.2	0.0	90.2

* Enlarged megaspore mother cells.

† Sum of per cent of resting megaspore mother cells and of uninucleate embryo sacs with crushed nucellar epidermis.

The mariola plant examined in this study showed considerable degeneration of ovular contents, and the breakdown began in the smallest ovules (table 6). Some undetermined environmental condition may have been the cause of so much degeneration, and perhaps the estimated 90.2 per cent of apomeiosis for this plant (table 7) is too high. The presence of aposporic reproduction is proved, nevertheless, by the occurrence of megaspore mother cells in so many classes of ovules, and by the enlargement of some of them to such an extent that the nucellar epidermis is crushed. According to Powers and Rollins (1945, table 4, p. 107), 18.2 ± 11.6 to 100 per cent of maternals occurred in F_1 progenies of mariola crossed with guayule. Some of these progenies also showed a fairly high per cent of aberrant hybrids.

Table 7 summarizes the megasporogenesis in all guayule lots and in the mariola plant used in the present study. The striking difference in the per cent of apomeiosis between the 36-chromosome plants and the other types of guayule has been sufficiently emphasized. This table gives no evidence that the degree of apospory in the polyploid plants is related to the chromosome numbers. Thus the 54-chromosome plant shows a higher per cent of apomeiosis than the average for the 72-chromosome plants, while the value for the 108-chromosome plant is below this average. As shown by Powers and Rollins's (1945) data, the per cent of apomixis may vary considerably within a given chromosomal group of guayule.

EMBRYO AND ENDOSPERM DEVELOPMENT IN GUAYULE AND MARIOLA

Table 8 illustrates the lack of definite relation between the development of the embryo and that of the endosperm in apomictic types of *Parthenium*—a lack already mentioned in the review of literature. This development was studied in all chromosomal types of guayule and in mariola. The headings concerning the contents of the embryo sacs in table 8 are best explained by reference to illustrations. Eggs and polar fusion nuclei of a 36-chromosome plant appear in plate 9, *B* and *C*. As was mentioned previously, in apomictic guayule the polar fusion nucleus becomes very large and lobed; it reminds one of the views given, in the literature, to illustrate triple fusion (plates 8, *C*; and 12, *C*). The term "polar fusion nucleus" in table 8 is used, therefore, with the reservation that possibly some of the structures listed under this term were products of triple fusion. Plate 9, *A*, shows an egg and some developing endosperm (the second endosperm nucleus appeared in the adjacent section); plate 11, *D*, an embryo and a polar fusion nucleus. The embryos in plate 12, *A* and *B*, were also accompanied by polar fusion nuclei. Incidentally, plate 12, *B*, shows an intact synergid to the left of the embryo. The other synergid was also intact,

TABLE 8

Relation between Embryo and Endosperm Development in Guayule and in Mariola

Lot no. in table 1	Approximate 2*n* number of chromosomes	Number of ovules examined	Percentages of ovules with the various contents						
			Egg and polar fusion nucleus	Egg and developing endosperm	Embryo and polar fusion nucleus	Embryo and developing endosperm	Polar fusion nucleus only	Developing endosperm only	Degenerated material
			Guayule						
4	36	74	28.4	0.0	1.3	66.2	0.0	0.0	4.1
5	36	111	25.2	0.0	0.0	55.0	0.0	19.8	0.0
7	54	108	11.1	0.0	17.6	63.0	0.9	1.9	5.5
8	54, 58, 72	103	2.9	0.0	7.8	42.7	7.8	8.7	30.1
10	72	93	1.1	0.0	9.6	55.9	14.0	5.4	14.0
12	72	91	8.8	2.2	12.1	42.9	18.7	3.3	12.0
13	72	95	3.2	0.0	33.7	46.2	0 0	5.3	11.6
14	108	47	10.6	0.0	17.0	27.7	25.5	6.4	12.8
			Mariola						
17	54	55	1.8	0.0	20.0	60.0	3.7	1.8	12.7

and the presence of both these structures is usually taken to indicate that the pollen tube had failed to enter the embryo sac. Figure 4, *K*, illustrates a belated initiation of endosperm in the presence of an embryo. Embryos and developing endosperm appear in plates 11, *A–C;* 12, *D;* and 13. The heading "polar fusion nucleus only" is illustrated diagrammatically in figure 4, *I;* "developing endosperm only" in figure 5, *E*. Because of degeneration, eggs or embryos are absent in embryo sacs appearing under the above two headings in table 8. If degeneration had progressed far enough to make the contents unidentifiable (fig. 4, *W*), the embryo sac was classified as "degenerated." Figures 4 and 5 show many of the variations in the manner of embryo and endosperm development in apomictic guayule.

TABLE 9

EMBRYO AND ENDOSPERM DEVELOPMENT IN 72-CHROMOSOME GUAYULE, VARIOUSLY POLLINATED

Number of days (collection no.) after pollination	Number of ovules examined	Percentages of ovules with the various kinds of embryo			Percentages of ovules with the various kinds of endosperm		
		Normal in size	Under-developed	None or degenerated	In stage of few to many cells	In stage of single nucleus	Degenerated
				Plant no. 49–3			
1	97	84.6	4.1	11.3	43.3	47.5	9.2
2	117	76.0	6.0	18.0	48.7	41.0	10.3
3	93	63.5	3.2	33.3	56.0	31.2	12.8
5	44	31.8	43.2	25.0	52.3	43.2	4.5
10	18	33.4	22.2	44.4	83.3	5.6	11.1
				Plant no. 427–4			
1	97	81.4	13.4	5.2	19.6	80.4	0.0
2	140	90.7	3.6	5.7	24.1	73.0	2.9
3	119	85.0	5.8	9.2	30.2	69.0	0.8
5–6	62	22.6	54.8	22.6	54.9	41.9	3.2
10	78	15.4	60.2	24.4	51.3	41.0	7.7

The material in table 8 was derived from open-pollinated plants. The degree of maturity of the achenes varied somewhat in the different lots, and this variation probably caused in part the differences in percentages of eggs and polar fusion nuclei in these lots.

Judging from table 8, the initiation of the embryo and endosperm are closely related in the predominantly sexual 36-chromosome guayule. This probability is indicated by the lack of views of eggs with developing endosperm and the scarcity of views showing an embryo with a polar fusion nucleus. Usually, when an embryo is present, there is also some developing endosperm. Abnormalities, however, occur in the 36-chromosome guayule. In lot 5 many embryo sacs contained developing endosperm, but no eggs or embryos; in lot 4 one embryo sac contained an embryo and a polar fusion nucleus, and several others had degenerated contents.

In contrast to the 36-chromosome guayule (table 8, lots 4 and 5), the predominantly apomictic types of *Parthenium* (table 8, lots 7–17) show many embryos not associated with developing endosperm and occasionally the endosperm begins to develop before the embryo. Many eggs and embryos degenerate, while the polar fusion nuclei or the endorsperm remain intact, at least temporarily; or the endosperm or its precursor also degenerate.

Thus the 36-chromosome guayule used in this study showed a close correlation between embryo and endosperm in their development, the former rarely appearing without the latter. In the other types of guayule and in the mariola, the embryo and the endosperm usually developed together; but often the former appeared first. The rather close correlation in development appears to be associated with the tendency toward normal sexuality in the 36-chromosome guayule; whereas the lack of such correlation in the other types of *Parthenium* represented in table 8 must be one of the phenomena of apomictic reproduction in these types.

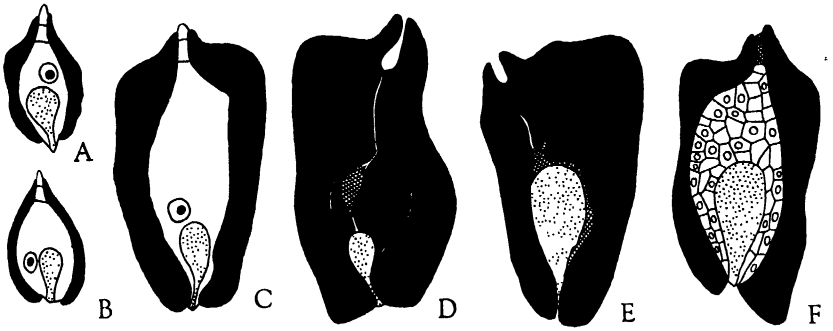
TABLE 10

EMBRYO AND ENDOSPERM DEVELOPMENT IN NONPOLLINATED 72-CHROMOSOME GUAYULE

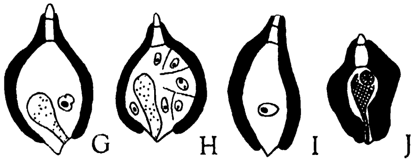
Number of days (collection no.) after anthesis	Number of ovules examined	Percentages of ovules with the various kinds of embryo			Percentages of ovules with the various kinds of endosperm		
		Normal in size	Under-developed	None or degenerated	In stage of few to many cells	In stage of single nucleus	Degenerated
Plant no. 49.3							
3	35	82.9	5.7	11.4	2.9	91.4	5.7
5-6	92	1.1	90.1	8.8	1.1	86.8	12.1
10	67	17.9	70.2	11.9	22.4	55.2	22.4
Plant no. 427.4							
3	69	89.8	8.7	1.5	0.0	98.5	1.5
5-7	58	0.0	100.0	0.0	3.4	96.6	0.0
10	35	0.0	88.6	11.4	40.0	60.0	0.0
12	40	0.0	65.0	35.0	25.0	50.0	25.0

Apomeiosis usually results in the formation of a diploid gametophyte with an unreduced egg capable of producing an embryo without fertilization. Powers and Rollins (1945) found proof of the common occurrence of such embryos in 72-chromosome guayule and in mariola in observing that maternal types predominated in the F_1 hybrid generation from such plants. Though initiated without fertilization, an apomictic embryo may fail to develop if pollination is excluded. In their comprehensive pollination studies, Powers and Rollins (1945, tables 1 and 2) first obtained some viable seed from emasculated and bagged flower heads of apomictic guayule. With subsequent refinement of methods, no viable seeds were obtained from nonpollinated flowers, and therefore these authors concluded that such seed was not formed in apomicts unless pollination occurred. They also found that self-pollination and intraspecific cross-pollination were about equally effective in inducing development of viable seed in apomicts.

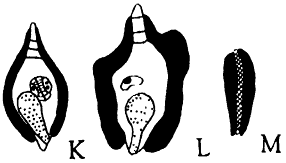
Besides the series of plants used by Powers and Rollins (1945) to obtain the data for their table 1, two plants, nos. 43–3 and 427–4, were similarly treated; and the material was sent to the present writer for a morphologic study on the



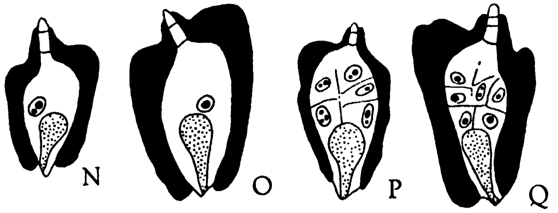
Nonpollinated, 3 to 12 days after anthesis



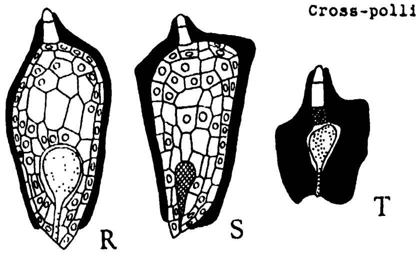
Cross-pollinated, 1 day



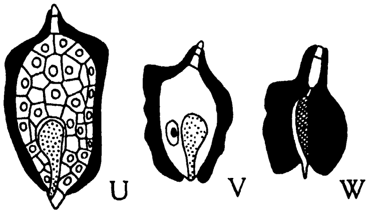
Self-pollinated, 1 day



Cross-pollinated, 2 days



Cross-pollinated, 3 days



Self-pollinated, 3 days

Fig. 4.—Diagrams of longitudinal sections of embryo sacs from 72-chromosome guayule plants that were used in pollination experiments. Six groups of embryo sacs appear in this figure, each from a differently treated group of flowers. The treatment and the number of days (collection no.) between pollination (or anthesis) and collection are given under each group of embryo sacs. In the diagrams the nutritive jacket is shown in solid black, the embryo is stippled, and the degenerating structures are cross-hatched. The endosperm or its precursor is represented by outlines of nuclei or of cells with nuclei; the antipodals by cell outlines. *A–E* and *G–W* are from plant 49–3; *F*, from plant 427–4. (All × 80.)

effect of pollination upon the development of apomictic embryos. The collections for this study were made at successive intervals after pollination (or after anthesis in the nonpollinated flowers). All treatments described by Powers and Rollins (1945) in their table 1, except treatment 3, were represented in the material sent to Davis. Tables 9 and 10 show the results of the morphologic study of this material. Since all the treatments involving pollination (open pollination, and controlled self- and cross-pollinations) yielded similar results (see also Powers and Rollins, 1945), the data from the variously pollinated

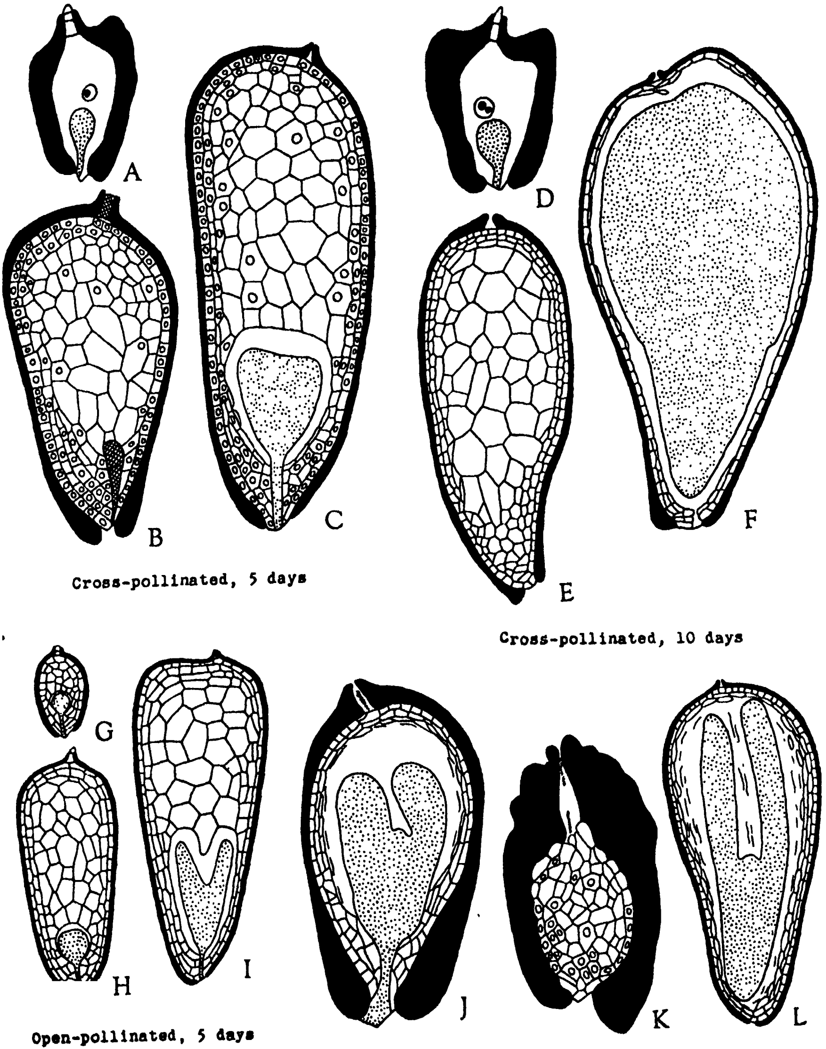


Fig. 5.—Diagrams of longitudinal sections of embryo sacs from 72-chromosome guayule plants that were used in pollination experiments. Details of presentation are as in figure 4, except that figure 5 shows only four groups of ovules. *A–F* are from plant 49–3; *G–L*, from plant 427–4. (*A–D*, *J*, and *K*, × 80; *E–I* and *L*, × 34.)

material were combined in table 9. Results with the nonpollinated flowers have been compiled in table 10.

Tables 9 and 10 give the data on embryo and endosperm separately. Apparently under all conditions of growth and pollination, embryo development in guayule lacks uniformity, especially in the apomictic types of plants. Shortly after anthesis, embryos tend to vary within a narrow size range; but later the variation is very great. To determine the normal size for an embryo at a given time after anthesis was therefore problematical; the estimate was made somewhat arbitrarily for tables 9 and 10. One to three days after pollination (table 9), the embryos were somewhat similar in size. Figures 4, *G*, *H*, *K*, and *L*, illustrate normal-sized embryos in collection 1 (made 1 day after pollination); the embryo in plate 11, *B*, however, was grouped with the eggs (or zygotes) among the underdeveloped embryos in collections 1. Normal embryos for collections 2 appear in figure 4, *N–Q*, and in plates 11, *C*, and 12, *B*. Plate 11, *A*, shows a small embryo of a collection 3; normal embryos for collections 3 are represented in plate 11, *D*, and figure 4, *R*, *T*, *U*, and *V*. The collections made 5 days after pollination contained such underdeveloped embryos as in plate 12, *A*, and in figure 5, *A* and *G*. The normal-sized embryos in these collections showed cotyledons in several stages of initiation and early development (plate 13, *A* and *B*, and fig. 5, *C*, *H*, and *I*). Plate 12, *D*, depicts an underdeveloped embryo from collection 7; figure 5, *D* and *J*, such embryos from collection 10. Embryos of normal size for the collections taken 10 days after pollination are shown in plate 13, *C*, and figure 5, *F* and *L*. These are full-sized or nearly so. "Degenerated embryos" occur in figures 4, *J*, *M*, *S*, *W;* 5, *B*, and in plate 14, *B;* embryos are absent in figures 4, *I;* 5, *E* and *K*, and in plate 16, *C*. The endosperm is "in stage of few to many cells" in figures 4, *H*, *K*, *P–S*, *U;* 5, *B*, *C*, *E–L;* and in plates 11, *A–C*, 12, *D*, and 13. It is "in stage of single nucleus" (probably mostly polar fusion nucleus) in figures 4, *G*, *I*, *L*, *N*, *O*, and *V;* 5, *A* and *D;* and plates 11, *D*, and 12, *A–C*. Endosperm was degenerated in embryo sacs like those in figure 4, *J*, *M*, *T*, and *W;* and plate 14, *B*.

According to table 9, in both plants 49–3 and 427–4 over 80 per cent of the ovules contained normal-sized embryos 1 day after pollination. Such embryos continued to predominate on the two following days. The remaining embryos were underdeveloped or degenerated. Despite the good start, however, many embryos stopped or slowed down their enlargement, so that 5 to 10 days after pollination the per cent of normal-sized embryos dropped perceptibly. On the last day of collection the predominant type became either the underdeveloped (plant 427–4) or the degenerated embryo (plant 49–3). In the first 3 days after pollination the endosperm lagged behind the embryos in development; but later it overtook them, in samples taken 5 to 10 days after pollination. The endosperm was less susceptible to degenerative changes than the embryo.

The degeneration of the embryos depends, at least partly, on the behavior of the endosperm. According to all observations thus far, if the endosperm does not develop, the embryo may start, but degenerates eventually. No embryos associated with a single nucleus in the primary endosperm cell were found to be larger than that in plate 14, *C*. Those underdeveloped embryos that are associated with an "endosperm in stage of single nucleus" (table 9) may therefore be expected to degenerate eventually. Some of the underdeveloped embryos that were imbedded in cellular endosperm may have continued development. The presence of normal-appearing endosperm, however, does not always assure continuous development of embryos: the latter frequently degenerate in the presence of good endosperm (figs. 4, *S*; 5, *B*, *E*, and *K*). The endosperm seems to depend less on the embryo than the embryo on the endosperm: the former may continue developing even if the embryo degenerates early or is not formed at all (fig. 5, *E*). Some of the endosperm developing without an embryo shows the usual thin cytoplasm of normal embryo sacs and develops large cells in the center of the sac, in contrast to the smaller cells near the periphery. Thus in figure 5, *E*, the endosperm without an embryo resembled in all respects that of the normal embryo sac in figure 5, *C*. Other endosperm developed abnormally, lacked differentiation in cell size, and had rather dense cytoplasm (fig. 5, *K*, and plate 16, *C*).

The final per cent of achenes with normal embryos in plant 49–3 approaches the average values for per cent of viable seed in the different pollination treatments performed by Powers and Rollins (1945, table 1); plant 427–4 shows a lower value. Both plants had a lower per cent of normal-sized embryos than the per cent of "seeds with cotyledons and presumably embryos" in the selfed plants of Powers and Rollins (1945, table 2). One cause of these discrepancies may be the relatively small number of achenes examined in the present study, as contrasted with the large numbers used by Powers and Rollins (1945). Second, since these authors harvested their seed at maturity (it was at least 2 months old), some of the underdeveloped embryos may have had time to complete their development.

. Judging by table 10, exclusion of pollination does not affect the initiation of embryos: 3 days after anthesis, over 80 per cent of ovules from nonpollinated flowers had embryos of normal size. In one plant (427–4) no embryo reached full size at the end of the experiment; in the other (49–3), 17.9 per cent developed into full-sized embryos or nearly so. The endosperm was delayed, as compared with that in the pollinated flowers, and ultimately developed in fewer ovules. That it was formed at all in the nonpollinated flowers may mean that the polar fusion nucleus itself is capable of giving rise to endosperm. On the other hand, according to Powers and Rollins (1945, p. 99), some accidental pollination might have occurred in this batch of material. Later an additional nonpollinated sample was investigated: heads of plant 593–I, which was also used in the fertilization studies (see "Material and Methods"), were emasculated and bagged. Among 55 mature achenes, 12.7 per cent had degenerated embryo sacs; 61.9 per cent contained embryos not larger than the one in plate 14, *C*; 1.8 per cent had good eggs, and 23.6 per cent degenerated eggs; 87.3 per cent showed single nuclei in the primary endosperm cells, and none had any developing endosperm. Figure 4, *A–F*, shows some ovules from the nonpollinated material used for table 10. These were taken the following number of days after anthesis: 4, *B*, 1 day; 4, *A*, *C* and *D*, 3 days; 4, *E*, 10 days; 4, *F*, 12 days. Plate 14, *C* and *D*, shows embryos and polar fusion nuclei in ovules collected 6 and 18 days after anthesis, respectively.

Judging from these pollination experiments, exclusion of pollination does not prevent the initiation of embryos; pollination is beneficial or perhaps even indispensable for endosperm development; and the absence of developing endosperm precludes complete development of the embryos. These data and the results of the pollination studies of Powers and Rollins (1945) permit a conclusion—namely, that apomeiosis leads to the initiation of an embryo without fertilization, but that pollination promotes endosperm development and is necessary for the formation of viable seed (pseudogamy). In the work of Powers and Rollins (1945), the maternal plants in the F_1 hybrid progenies of apomictic guayule—and consequently also the embryos from which these maternals grew—had the same numbers of chromosomes as the mother plants. Hence in apomictic guayule, apospory is followed by unreduced pseudogamy.

The ultimate cause of degeneration of so many embryos in pollinated flowers has not been revealed by the present study. The lack of endosperm development—and with it the cessation of growth of any embryos initiated—may result from some deficiency in fertilization in the apomicts. This suggestion is borne out by the common occurrence of normally developing endosperm in the open-pollinated predominantly sexual 36-chromosome plants (table 8). Many embryos or eggs, however, degenerate in the presence of developing endosperm; such degeneration occurs in both the apomicts and the sexual guayule (table 8, ninth column). Powers and Rollins (1945) also found comparatively low germination in seeds of 36- and 72-chromosome guayule. Though environmental conditions influence the quality and quantity of seed produced by guayule (Whitehead and Mitchell, 1943; Mitchell *et al.*, 1944), internal factors must be involved also.

Powers and Rollins (1945) observed a pronounced difference in the degree of self-compatibility in the 36- and 72-chromosome plants. The former were found highly self-sterile; in the latter, self-pollination and cross-pollination within the species were found similarly effective in producing viable seed. In fact, selfing sometimes gave more good seed than intraspecific crosses (Powers and Rollins, 1945, table 1, p. 100). Interspecific crosses, however, consistently gave higher amounts of viable seed than selfing. Some material was available for a morphologic study of self-sterility and self-fertility. Heads with a total of 30 flowers of the 36-chromosome plant of lot 5 (table 1) were emasculated and selfed. Fourteen days after pollination, none of these contained embryos or endosperm; a few had eggs and polar fusion nuclei; and the rest showed only degenerated contents. The 36-chromosome plant of lot 4 bloomed for a time at Davis, in a greenhouse where no other guayule plant was flowering. The 120 oldest achenes collected after this blooming period contained the following: 97.5 per cent of eggs and polar fusion nuclei; 1.7 per cent of embryos and developing endosperm; 0.8 per cent of embryo sacs younger than 7-celled. Similar achenes from open-pollinated material had over 60 per cent of embryo sacs with embryos and developing endosperm (table 8). Plate 9, *C*, shows an egg and a polar fusion nucleus from an old nonpollinated flower, as contrasted with those just ready for fertilization in plate 9, *B*. Self-sterility is well illustrated by the observations just mentioned and the 1.7 per cent of embryos in one of the self-pollinated samples might have been apomictic. As a further check on apomixis in 36-chromosome guayule, some flower heads were emasculated and bagged. The 36 ovules collected from these heads had neither embryos nor endosperm.

From 5 to 10 days after pollination, plant 427–4 showed, upon self-pollination, 82.9 per cent of embryos and 45.1 per cent of sacs with developing endosperm in 82 ovules; and upon cross-pollination (intraspecific cross), 67.2 per cent of embryos and 63.5 per cent of sacs with developing endosperm in 58 ovules. Plant 49–3 was for a time the only guayule growing in a green house at Davis. The 133 fully developed achenes collected at this time had 54.1 per cent of full-sized embryos, 15.8 per cent of underdeveloped embryos, and 54.9 per cent of sacs with developing endosperm. The two 72-chromosome plants above obviously showed a high degree of self-compatibility.

To check whether or not apomictic embryos tend to start developing before the normal ones, the development of the embryo sac was related to that of the pistillate flower, and these data were compared for the sexual and apomictic types of guayule. Four stages of flower development were used (tables 11, 12, and 13): in the first and youngest, the ray of the corolla was still concealed within the involucral bracts; in the second, it was more or less exserted; in the third, the stigmas of the pistillate flowers were fully extruded and expanded; in the fourth, they were brown and withered.

TABLE 11

RELATION BETWEEN DEVELOPMENT OF THE PISTILLATE FLOWER AND DEVELOPMENT OF THE EMBRYO SAC IN THE 36-CHROMOSOME GUAYULE OF LOT 4 IN TABLE 1

Contents of ovules	Percentages of different ovule contents in flowers of various developmental stages*			
	Ray concealed within bracts	Ray more or less exserted	Stigmas expanded	Stigmas necrosed
Embryo sacs younger than 7-celled	33.9	0.0	0.0	0.0
Embryo sacs with eggs or zygotes	61.2	94.9	68.1	28.4
Embryo sacs with embryos	0.0	3.4	26.4	67.5
Embryo sacs with polars or polar fusion nuclei	61.2	91.5	62.5	29.7
Embryo sacs with developing endosperm	0.0	6.8	32.0	66.2
Degenerated embryo sacs	4.9	1.7	5.5	4.1
Total of 7-celled and older embryo sacs†	61.2	98.3	94.5	95.9

* Numbers of ovules examined for the respective data columns reading from left to right are: 121, 59, 72, and 74.

† Total of embryo sacs with eggs, zygotes, and embryos; or total of embryo sacs with polars, polar fusion nuclei, and developing endosperm.

As table 11 shows, in a 36-chromosome plant the maturation of the female gametophyte occurs before the pistillate flowers emerge from the bracts. When the ray corollas become exserted, the flowers contain no embryo sacs younger than 7-celled. Judging by the first appearance of the embryos, pollination and fertilization occur before the stigmas are fully expanded. In some ovules the endosperm develops slightly before the embryos—a fact evidenced in table 11, by the higher per cent of embryo sacs with developing endosperm than of sacs with embryos. In the oldest achenes this lag disappears. (Among the old achenes, 1.3 per cent contained embryos without endosperm, as shown in table 8.) Some embryo sacs in all four kinds of flowers had degenerated contents.

The polyploid types of guayule differed from the 36-chromosome plants in details of development in the embryo sacs, but embryos were initiated in flowers at the same stage of development as in the sexual guayule (tables 12 and 13). The endosperm began to develop somewhat later in the 72-chromosome plants. According to these data, the apomictic embryos do not appear earlier than the normal ones.

The lack of orderly sequence in the development of the embryo sac in the polyploid guayule, which was discussed earlier in this paper, is also apparent from tables 12 and 13: embryo sacs younger than 7-celled and unfused polars occurred in flowers of all stages. In the 36-chromosome plant (table 11) only the youngest flowers contained immature gametophytes and polars. (The latter were so few that they were not counted separately from the polar fusion nuclei in table 11.) Among the unidentified embryo sacs (tables 12 and 13), many showed abnormal structure. Sometimes they had extra nuclei; or they consisted of several uni- or binucleate cells, as though all four megaspores were developing into embryo sacs; or one cell had several nuclei, and another had one. Parts of some sacs resembled those of a 7-celled embryo sac, but seemed incompletely differentiated. The mixed polyploid lot (table 13) had more of these abnormal and also of degenerating embryo sacs than the 72-chromosome guayule (table 12). As a rule, degeneration was more pronounced in the polyploid plants (tables 12 and 13) than in the diploid (table 11).

TABLE 12

RELATION BETWEEN DEVELOPMENT OF THE PISTILLATE FLOWER AND DEVELOPMENT OF THE EMBRYO SAC IN 72-CHROMOSOME GUAYULE

(Combined data from lots 9 and 10 in table 1)

Contents of ovules	Percentages of different ovule contents in flowers of various developmental stages*			
	Ray concealed within bracts	Ray more or less exserted	Stigmas expanded	Stigmas necrosed
Embryo sacs younger than 7-celled	65.6	23.8	5.8	0.0
Embryo sacs with eggs or zygotes	11.0	37.3	50.3	3.7
Embryo sacs with embryos	0.0	0.4	7.2	63.1
Embryo sacs with degenerated or unrecognizable eggs or embryos	10.9	16.0	20.9	20.1
Embryo sacs with polar nuclei	16.4	19.3	7.9	0.5
Embryo sacs with polar fusion nuclei	5.5	34.4	56.8	31.3
Embryo sacs with developing endosperm	0.0	0.0	13.7	55.1
Entirely degenerated embryo sacs	10.9	16.4	12.2	11.7
Unidentified embryo sacs	1.6	6.1	3.6	1.4
Total of identified 7-celled and older embryo sacs†	21.9	53.7	78.4	86.9

* Numbers of ovules examined for the respective data columns reading from left to right are: 128, 244, 139, and 214.

† Total of embryo sacs with eggs, zygotes, embryos, and unrecognizable eggs and embryos; or total of embryo sacs with polar nuclei, polar fusion nuclei, and developing endosperm.

The degenerative changes in the embryo sac of guayule merit a brief characterization. The most striking feature is the hyperplastic development of the nutritive jacket, similar to the development described by Brink and Cooper (1940) and Cooper and Brink (1940) in their studies on somatoplastic sterility. The normal nutritive jacket is one cell thick. In hyperplastic development it becomes multiseriate through a series of periclinal divisions (plates 12, *A*; 14, *B–D*; 15, *A*; and 16). Through this growth the nutritive jacket may more or less occlude the lumen of the embryo sac (figs. 4, *D*, *E*, *M*, *T*, and *W*;

5, *K;* plates 14, *B;* and 16). The degree of thickening of the nutritive jacket occurs without relation to any obvious morphologic features of the embryo sac. As figures 4 and 5 show, either thinner or thicker nutritive jackets may enclose embryo sacs with and without embryos and with and without developing endosperm. In plate 14, *B*, the nutritive jacket was occluding a degenerating embryo; in plate 16, *A*, on the other hand, the embryo was still actively dividing, especially on the periphery, as though resisting the encroachment by the highly hyperplastic nutritive jacket. In both these embryo sacs the endosperm (or its precursor) had previously degenerated. The antipodals, however, were still intact in the embryo sac in plate 14, *B*. In plate 16, *B*, the hyperplastic nutritive jacket had enveloped a rather large embryo of abnormal shape and had almost separated it from the endosperm. The thick jacket in plate 16, *C*, surrounds an embryo sac without an embryo but with some abnormal endosperm. Plate 14, *C* and *D*, characterize embryo sacs from nonpollinated flowers of 72-chromosome guayule. Six days after anthesis, the jacket cells still had dense cytoplasm (plate 14, *C*); but 18 days after anthesis, the cytoplasm was thin, and the jacket apparently had ceased to grow (plate 14, *D*). The embryo sacs in nonpollinated and self-pollinated flowers of 36-chromosome guayule, which contained no embryos, were frequently characterized by thickened nutritive jackets. Some, however, had thin jackets that were breaking down. Degenerative changes may occur during the early megasporogenesis. For example, plate 14, *A*, illustrates collapsed nucellar epidermis (cells with thin cytoplasm in the center of the picture) enclosed by a nutritive jacket that is somewhat thickened in the region toward the micropyle (below in the photograph). Such early degeneration may be followed by a perceptible thickening of the nutritive jacket (fig. 4, *M*). Apparently many kinds of abnormalities in embryo-sac development may induce hyperplastic proliferation in the nutritive jacket.

TABLE 13

RELATION BETWEEN DEVELOPMENT OF THE PISTILLATE FLOWER AND DEVELOPMENT OF THE EMBRYO SAC IN MIXED POLYPLOID GUAYULE COLLECTION OF LOT 8 IN TABLE 1

Contents of ovules	Percentages of different ovule contents in flowers of various developmental stages*			
	Ray concealed within bracts	Ray more or less exserted	Stigmas expanded	Stigmas necrosed
Embryo sacs younger than 7-celled	21.1	27.6	21.1	5.2
Embryo sacs with eggs or zygotes	43.5	42.9	28.4	17.0
Embryo sacs with embryos	0.0	1.0	2.1	41.2
Embryo sacs with degenerated or unrecognizable eggs or embryos	4.4	4.0	7.4	8.8
Embryo sacs with polar nuclei	4.4	3.0	9.5	3.1
Embryo sacs with polar fusion nuclei	43.5	43.9	24.2	26.3
Embryo sacs with developing endosperm	0.0	1.0	4.2	37.6
Entirely degenerated embryo sacs	23.3	19.4	28.4	20.1
Unidentified embryo sacs	7.7	5.1	12.6	7.7
Total of identified 7-celled and older embryo sacs†	47.9	47.9	37.9	67.0

* Numbers of ovules examined for the respective data columns reading from left to right are: 90, 98, 95, and 194.

† Total of embryo sacs with eggs, zygotes, embryos, and degenerated eggs and embryos; or total of embryo sacs with polar nuclei, polar fusion nuclei, and developing endosperm.

FERTILIZATION EXPERIMENTS WITH GUAYULE

The fertilization studies were designed to compare the phenomena of fertilization in a 36- and a 72-chromosome guayule plant. Both plants were pollinated with pollen from 72-chromosome guayule. (See "Material and Methods.") The flower heads were emasculated before the ray-florets had emerged from the involucral bracts and been bagged. The flowers were pollinated when the stigmas had expanded and appeared receptive. The collections were made at emasculation time; at pollination, before the pollen was applied to the stigmas; and every hour for 6 hours thereafter. The choice of the earliest time for collection after pollination was based on the report by Dianowa and her coworkers (1935) that fertilization in *Parthenium argentatum* occurred 1 hour after pollination. In the experiment with the 72-chromosome plant, the first collection was made ½ hour after pollination. Tables 14 and 15 give the numbers of ovules in each collection. Because of technical difficulties, the pollinations and collections had to be repeated on different days. In consequence, as will be indicated later, some differences occurred in the flowers treated.

The results of the fertilization experiments with 36-chromosome guayule, are given in table 14. As a rule, indications of accomplished fertilization were obvious; but the early stages of sperm entry into the embryo sac were not observed. Even the collections made 1 hour after pollination (table 14) showed the male nucleus within the egg, more or less fused with the nucleus of the latter; and the second sperm nucleus was at this time fused with the polar fusion nucleus. The wormlike male nuclei described by Gerassimova (1933–34) for *Crepis capillaris* were not recognized. Possibly the stages studied were too old to indicate the original appearance of the sperms when they occur in the pollen tube or emerge from the latter. All male nuclei were of ordinary appearance and resembled those depicted by Land (1900) for *Erigeron*. Plate 10 illustrates some typical evidences of fertilization in 36-chromosome guayule. In plate 10, *A*, the egg nucleus (with the distinct nucleolus) is in contact with the male nucleus. The primary endosperm nucleus is lobed because of recent fusion with a male nucleus; it also shows two small nucleoli beside the large nucleolus of the polar fusion nucleus. Remnants of the pollen tube, in the form of a dark streak, partly envelop the egg. These remnants appear massive in the view in plate 10, *B*. The egg in 10, *B*, contained a male nucleus; but the latter came into view at a lower focus than the image in plate 10, *B*. The endosperm nucleus in this embryo sac, though not lobed, contained two extra small nucleoli derived from the male nucleus. (Only one is in focus in plate 10, *B*.) Plate 10, *C*, shows parts of one pollen tube in the micropyle and in the embryo sac. The egg in this sac was fertilized. As a rule one or both synergids were unmistakably broken down, in the presence of signs that a pollen tube had entered the embryo sac. Synergids, however, may present a disorganized aspect in unpollinated material; moreover, they are easily damaged in the preparation of the material for slide making. Their appearance, therefore, was not used to identify the occurrence of fertilization.

The eggs and the endosperm (or its precursor) were classified separately in table 14. The values for the eggs add up to 100, as do those for the endosperm. In addition, a separate item gives the per cent of ovules that showed remnants of pollen tubes. The latter were counted only if they were positively identified and were clearly distinguishable from disintegrating synergids. If the embryo sac showed no evidence of fertilization, it fell into the group of sacs containing unfertilized eggs and polar fusion nuclei. If the male nucleus was observed within the egg (in no view did it occur outside), the egg was classed as fertilized. Apparently after the complete fusion of the sperm and egg nuclei, the evidence of accomplished fertilization becomes obscured until the zygote begins to divide. Nowhere in this study, therefore, has a separate classification of eggs and zygotes been attempted. If the egg carried no clear indication of having been fertilized, but other marks of fertilization were present in the same embryo sac (fertilization of the polar fusion nucleus, remnants of pollen tube), the egg was grouped with the unidentified ones. Embryos were easily recognized.

TABLE 14

FERTILIZATION DATA ON 36-CHROMOSOME GUAYULE POLLINATED WITH POLLEN FROM A 72-CHROMOSOME GUAYULE

Contents of ovules	Percentages of different ovule contents*		Percentages of different ovule contents at the given number of hours after pollination*					
	At time of emasculation	At time of pollination	1	2	3	4	5	6
Unfertilized eggs	100.0	100.0	65.4	50.0	20.0	14.8	10.3	3.7
Fertilized eggs	0.0	0.0	11.5	5.0	46.7	63.0	75.9	66.7
Unidentified eggs	0.0	0.0	23.1	45.0	20.0	11.1	13.8	29.6
Embryos	0.0	0.0	0.0	0.0	13.3	11.1	0.0	0.0
Polar fusion nuclei	100.0	100.0	65.4	50.0	20.0	14.8	10.3	3.7
Primary endosperm nuclei	0.0	0.0	7.7	10.0	30.0	63.0	86.2	66.7
Developing endosperm	0.0	0.0	0.0	0.0	16.7	11.1	0.0	0.0
Unidentified endosperm	0.0	0.0	26.9	40.0	33.3	11.1	3.5	29.6
Pollen tubes	0.0	0.0	34.6	50.0	63.3	70.4	89.8	63.0

* Numbers of ovules examined for the respective data columns reading from left to right are: 26, 23, 26, 20, 30, 27, 29, and 27.

As was mentioned above, polar fusion nuclei occurred in embryo sacs that showed no evidence of fertilization. Primary endosperm nuclei were those that indicated recent triple fusion. In the literature the presence of extra nucleoli in the endosperm nucleus is usually assumed to be an evidence of fertilization. The study of the 36-chromosome material supports this assumption. Upon maturation the embryo sac contains a nonlobed polar fusion nucleus with a single large nucleolus (plate 9, *B*). Even if fertilization is omitted and the embryo sac ages, the polar fusion nucleus retains essentially the same characteristics (plate 9, *C*). After a recent triple fusion, the nucleus of the primary endosperm cell commonly shows lobing (plate 10, *A*) and contains extra nucleoli (plate 10, *A* and *B*). Later, however, the nucleus rounds off, the nucleoli seem to fuse, and the nature of the nucleus in the primary endosperm cells becomes uncertain until the endosperm begins to develop. If the nucleus in the primary endosperm cell showed no certain evidence of triple fusion, but was associated with some other signs of fertilization in the same embryo sac (remnants of pollen tube, male nucleus within the egg), it was classified as "unidentified endosperm." The developing endosperm (that is, endosperm consisting of more than one cell) was recognized as easily as the embryos.

Table 14 shows that at emasculation and pollination the embryo sacs were mature; they contained eggs and polar fusion nuclei. One hour after pollination about one third of the embryo sacs contained pollen tubes, and several showed fertilized eggs and primary endosperm nuclei. The per cent of embryo sacs with pollen tubes and with primary endosperm nuclei continued to increase up to and including the fifth collection after pollination. Excepting the second hourly collection (collection made 2 hours after pollination), the samples showed a similar successive increase in per cent of fertilized eggs, up to and including the fifth hourly collection. The drop in per cent of fertilized eggs the second hour may be only apparent: the first collection after pollination consisted of two samples prepared 5 days apart. The first sample that was prepared on the same day as the second hourly collection showed no fertilized eggs or primary endosperm nuclei and only a few pollen tubes. Similarly the third and fourth hourly collections consisted of two samples each, and those made later contained all the embryos and all the developing endosperm. Apparently the flowers used in the later set of treatments were farther advanced in their development than those used 5 days earlier. The drop in the per cent of views indicating fertilization in the collections made 6 hours after pollination may have resulted from natural variability (the fifth and sixth hourly collections were prepared on the same day); or it may have been caused by the obliteration of the evidences of fertilization, through the complete fusion of male and female nuclei and the disintegration of pollen-tube remnants. Though no developing endosperm was present in the last collection, 18.6 per cent of the primary endosperm nuclei were in division.

TABLE 15

FERTILIZATION DATA ON 72-CHROMOSOME GUAYULE POLLINATED WITH POLLEN FROM ANOTHER 72-CHROMOSOME GUAYULE PLANT

Contents of ovules	Percentages of different ovule contents*		Percentages of different ovule contents at the given numbers of hours after pollination*						
	At time of emasculation	At time of pollination	½	1	2	3	4	5	6
Unfertilized eggs	57.2	40.0	54.6	50.0	49.0	65.4	42.5	25.8	23.6
Fertilized eggs	0.0	0.0	0.0	0.0	0.0	1.9	0.0	3.2	7.9
Degenerated eggs	9.5	14.3	13.6	14.0	17.6	7.7	15.0	3.2	23.7
Embryos	0.0	20.0	9.1	18.0	5.9	1.9	0.0	6.5	2.6
Polars or polar fusion nuclei	66.7	74.3	77.3	82.0	68.6	75.0	57.5	38.7	55.2
Primary endosperm nuclei	0.0	0.0	0.0	0.0	0.0	1.9	0.0	0.0	0.0
Developing endosperm	0.0	0.0	0.0	0.0	3.9	0.0	0.0	0.0	2.6
Young or degenerated embryo sacs	33.3	25.7	22.7	18.0	27.5	23.1	42.5	61.3	42.2
Pollen tubes	0.0	0.0	0.0	0.0	5.9	7.7	2.0	16.1	15.5

* Numbers of ovules examined for the respective data columns reading from left to right are: 42, 35, 22, 50, 51, 52, 40, 31, and 38.

Regardless of these variations, table 14 clearly shows that fertilization normally occurs in 36-chromosome guayule. This observation agrees with those made previously on the predominantly normal sequence in the megasporogenesis and embryo-sac development and on the close relation between endosperm and embryo development in this type of guayule. Obviously, normal sexual reproduction predominates in the 36-chromosome guayule used in the present study.

The 72-chromosome material (table 15) presents a different picture with regard to fertilization. Before table 15 is analyzed, the times of collection of the material must be indicated. The experiments with the 36-chromosome plant were carried out during the first week of April; those with the 72-chromosome plant during the last week in June of the same year. Both plants were located in a greenhouse at Salinas. There were two series of collections from the 72-chromosome plant. The first, prepared on June 23, consisted of collections from emasculation time up to and including 6 hours after pollination. No collection was made ½ hour after pollination in this series. The second series, prepared on June 30, contained collections from emasculation time up to and including 4 hours after pollination. The ovules of the second series proved to be more advanced than those of the first and contained most of the embryos recorded in table 15, but most of the pollen tubes occured in the first collection. Only one embryo sac showed both an embryo and remnants of a pollen tube.

As compared with the 36-chromosome plant, the 72-chromosome guayule showed little evidence of accomplished fertilization. There were few embryo sacs with remnants of pollen tubes and very few obviously fertilized eggs or primary endosperm nuclei. Undoubtedly these results are related to the tendency toward apomictic reproduction in 72-chromosome guayule. The particular plant used in the fertilization experiments was highly apomictic. According to a personal communication from Dr. R. C. Rollins, among 48 classifiable plants in the F_1 generation from this guayule plant, crossed with mariola, 95.84 per cent were maternals and only 4.16 per cent were hybrids. The latter could only have come from fertilized eggs. For proper development, however, embryos must be accompanied by developing endosperm—a product of triple fusion. Although several fertilized eggs were found in the ovules used for table 15, the evidence for the occurrence of triple fusion was extremely weak. The chief difficulty in the recognition of triple fusion in 72-chromosome guayule is caused by the peculiar morphology of the polar fusion nucleus. As was mentioned previously, this nucleus is large and lobed and mostly contains several nucleoli. Figure 6, *H–J*, illustrates three sections of such a nucleus. It occurred in an ovule containing an egg and two good synergids, which was collected 3 days after pollination. That the peculiar characteristics of the polar fusion nucleus are not associated with fertilization is proved by the occurrence of large, lobed, multinucleolate nuclei in samples collected at times of emasculation and pollination (table 15) and in flowers that were left entirely unpollinated (table 10). If a sperm nucleus were to occur in contact with such a nucleus, its distinction from one of the lobes of the polar fusion nucleus would probably be difficult. In one instance only (see the third hourly collection in table 15) the view seemed to indicate triple fusion (fig. 6, *F* and *G*). In this view there appeared to be two polars, each with a single nucleolus, partly fused with a sperm nucleus that had three small nucleoli. If such a nuclear configuration had occurred in a 36-chromosome plant, it would have been regarded as evidence of triple fusion; but in a 72-chromosome guayule its identity remains somewhat uncertain. Kokieva (1932) has described triple fusion and has even suggested that two sperm nuclei may fuse with the polar fusion nucleus; but the illustrations that are intended to support her interpretations resemble pictures of the enlarged and lobed polar fusion nuclei. Further studies are necessary to reveal the origin of endosperm in apomictic guayule—particularly endosperm that develops in association with apomictic embryos.

Besides indicating infrequency of fertilization in the 72-chromosome plant, table 15 gives other data that agree with the previously observed peculiarities in reproduction of apomictic guayule. The embryo sac matures unevenly. Some sacs younger than the 7-celled occurred in all stages of development; polars were still present in the collection made at emasculation; and a considerable number of sacs were undergoing degeneration. Embryos occurred before pollination, and most of the embryos recorded in table 15 were not associated with developing endosperm.

SOMATIC APOSPORY IN GUAYULE

A variable, but usually small, per cent of ovules of guayule show one, rarely two or three embryo sacs besides the one in normal position (plate 15, *B–D*). These extra sacs are located almost invariably at the antipodal end of the sac that is in normal position, and typically the two sacs are connected with each other through their antipodals (plate 15, *C*). Sometimes the extra sac is entirely outside the one located normally (plate 15, *C*); or it occurs within the latter (lower supernumerary sac in plate 15, *D*); or it appears to emerge from the cavity of the sac in normal position by breaking through the nutritive jacket of the latter (plate 15, *B*). The supernumerary sacs have no nutritive jackets of their own and often are surrounded by crushed cells (plate 15, *B*). Antipodals, eggs, synergids, embryos, polars, polar fusion nuclei (usually lobed), but never any developing endosperm, were observed in the extra embryo sacs. In early stages of development these sacs may be uni-, bi-, or tetranucleate. Sometimes the organization of an extra sac is not definable.

The frequency of the supernumerary embryo sacs varied from a fraction of 1 per cent to about 20 per cent in the material used in the present study. They were found in 36-, 54-, 72-, and 108-chromosome guayule and in mariola. Apparently their occurrence does not depend on the condition of the sac that is in normal position. Among 369 ovules in the variously pollinated material from plant 49–3 (table 9), 19.2 per cent showed supernumerary embryo sacs. The sacs in normal position that were associated with the supernumerary were grouped as follows: 35.2 per cent had developing endosperm; 38.0 per cent had polar fusion nuclei; 26.8 per cent were degenerating. Plate 15, *B*, shows a degenerating embryo and a hyperplastic nutritive jacket in the embryo sac located normally; in plate 15, *C*, the sac that is in normal position has developing endosperm and an ordinary nutritive jacket.

According to the literature (see "Review of Literature"), supernumerary embryo sacs like those in guayule are commonly derived from somatic cells, and their formation is referred to as somatic apospory. Unless very early stages of somatic apospory are available, its origin is difficult to ascertain, because the growing aposporic cell crushes and displaces the adjacent cells and thus markedly changes its original environment. Two features are particularly characteristic of the supernumerary embryo sacs in guayule: they appear late in the ontogeny of the sac that is in normal position, and arise at its antipodal end. Rarely is a supernumerary sac associated with one (located normally) that has not reached the 7-celled stage (fig. 6, *E*). Some figures support this observation. In a collection from plant 49–3, the flowers were divided into three classes: (1) 283 flowers that contained megaspore mother cells to just matured 7-celled embryo sacs; (2) 95 flowers with fully expanded stigmas that had eggs and young embryos; (3) 562 flowers taken 1 to 10 days after anthesis. The following percentages of flowers in each class contained supernumerary embryo sacs: class 1, 0.0 per cent; class 2, 9.5; class 3, 18.5.

Judging from the data given above, the early stages of somatic apospory must be sought in connection with 7-celled embryo sacs in normal position. Many views seemed to suggest that the antipodals were involved in the formation of the extra aposporic sacs: some antipodals appeared unusually large and were protruding into the chalazal region. In figure 6, *B*, the upper antipodal is enlarged, as contrasted with the upper antipodal in figure 6, *A*. The interpretation that antipodals develop into embryo sacs has been made in the literature. Chamberlain (1895) found extra embryo sacs in *Aster Novae-angliae* closely resembling those of guayule in morphology and in relation to the normally placed embryo sacs. He thought they arose from antipodal cells, but today a search in the guayule material reveals views that require a different interpretation. The antipodal cells, even if they enlarge, usually have rather dense cytoplasm (fig. 6, *B*). According to Stebbins and Jenkins (1939), however, aposporic cells are characterized by high degree of hydration. Such strongly vacuolated and obviously enlarging cells were found at the antipodal end of some embryo sacs in guayule. They appeared to be in proper position to produce a supernumerary embryo sac characteristic of guayule (fig. 6, *C*) and, through intermediate stages of binucleate and tetranucleate embryo sacs (fig. 6, *D*), were connected with the mature supernumerary embryo sacs. In their enlargement they crushed the adjacent cells (fig. 6, *D*) and perhaps the antipodals also. Views like those in plate 15, *B* and *D*, indicate that the cell forming the aposporic sac is not always located outside the embryo sac in normal position. Perhaps cells of the nucellar epidermis or nutritive-jacket cells produce sacs that intrude into the sacs in normal position (plate 15, *D*) or break the continuity in the nutritive jacket (plate 15, *B*). The present study therefore suggests that the supernumerary embryo sacs in *Parthenium* arise from some somatic cells located near the antipodals of the normally placed embryo sac.

No supernumerary embryo sac has been found to contain developing endosperm, and many were degenerating. Probably somatic apospory rarely results in the production of viable embryos. More than one embryo sometimes occurs in one achene in guayule. The extra embryo may arise not only through somatic apospory, but also in some other way. Achenes containing two ovules have occasionally been found. Sometimes two embryos occur in the same embryo sac that is in normal position; views of dividing synergids (plate 15, *A*) suggest that the second embryo could arise from a synergid. Sometimes the pair of embryos appear to have arisen through lobing of a single embryo. Two megaspore mother cells enclosed within the same nucellar epidermis occur in some ovules, but usually one is much smaller and shows signs of being crowded out by the other (fig. 2, *H* and *I*). All these phenomena—somatic apospory, synergid embryos, double ovules, and double megaspore mother cells—are rather infrequent. The main features characterizing the apomixis in the *Parthenium* material used in this study are generative apospory and pseudogamy.

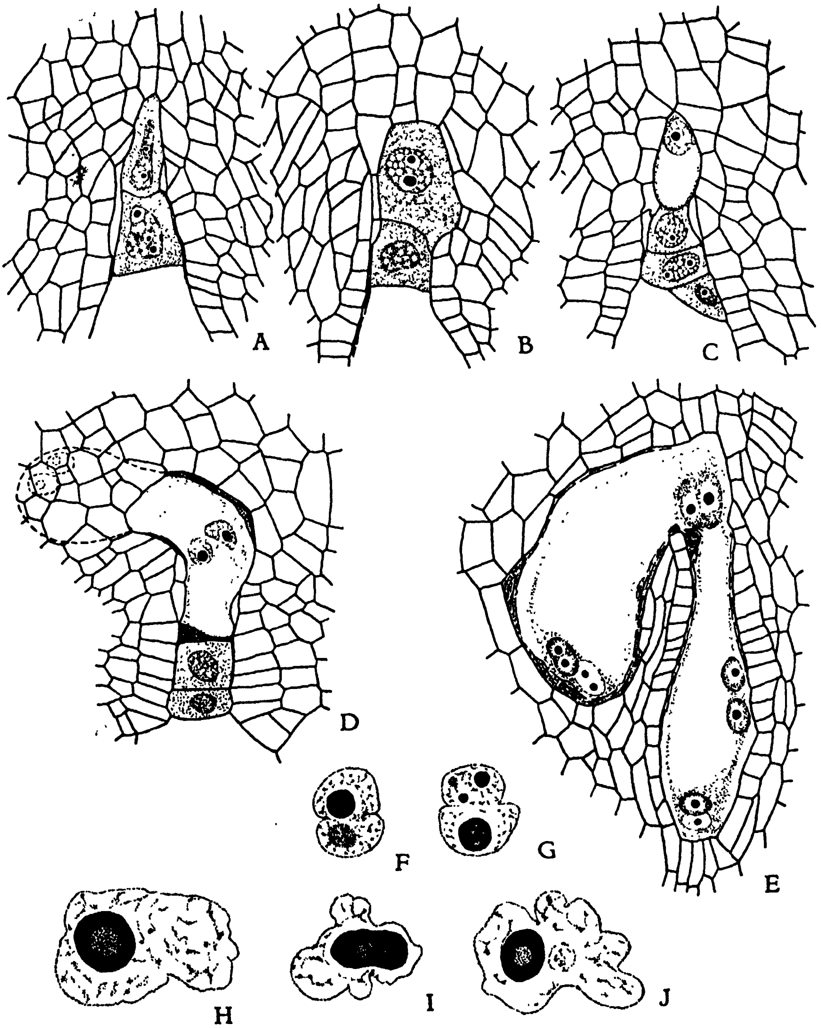


Fig. 6.—*A–D*, Antipodals (stippled throughout) and some adjacent ovular cells. In *B* the upper of the two antipodals is somewhat enlarged. In *C* a somatic cell next to the upper of the three antipodals is differentiating into an aposporic embryo sac. In *D* the aposporic sac above the antipodals has four nuclei and has crushed some ovular cells and apparently also one antipodal. *E*, Two tetranucleate embryo sacs connected with each other. The sac to the left might have arisen through somatic apospory. *F*, *G*, Two sections of the same group of three partly fused nuclei; one polar in *F*, above; second polar in *F* and *G*, below; and a sperm nucleus in *G*, above. *H*, *I*, *J*, Three sections of the same lobed polar fusion nucleus. All these drawings are from 72-chromosome guayule. (*A–E*, ×357; *F–J*, ×850.)

REPRODUCTION IN PARTHENIUM HYSTEROPHORUS

To obtain, early in this study, a clear picture of the differences between normal and apomictic types of *Parthenium*, the annual *P. hysterophorus* was used as a plant known to have sexual reproduction. Table 16 gives the data obtained with this plant. The flowers were divided into small, medium, and large according to the degree of development of the achenes and corollas. The stages of embryo-sac development were recorded for each flower as in the study of guayule. *P. hysterophorus* showed normal sequence of megasporogenesis and gametophyte development. As table 16 shows, all the megaspore mother cells were dividing (and all the divisions were meiotic), tetrads of megaspores were numerous (but dyads were few as in 36-chromosome guayule), and all uninucleate embryo sacs were associated with crushed megaspores. With the increase in flower size, the young stages were disappearing, and the embryo sacs matured. The polars fused during this maturation. The embryos were all associated with developing endosperm. Some embryo sacs were degenerated, apparently after they had reached the 7-celled stage.

TABLE 16

RELATION BETWEEN FLOWER SIZE AND EMBRYO-SAC DEVELOPMENT IN *Parthenium hysterophorus* L.

(Combined data from lots 15 and 16 in table 1)

Contents of ovules	Percentages of different ovule contents in flowers of various sizes*		
	Small	Medium	Large
Dividing megaspore mother cells	13.3	0.0	0.0
Dyads	1.7	0.0	0.0
Tetrads of megaspores	50.0	0.7	0.0
Uninucleate embryo sacs with crushed megaspores	13.3	0.0	0.0
Binucleate embryo sacs	11.7	0.7	0.0
Tetranucleate embryo sacs	6.7	3.4	0.7
Seven-celled embryo sacs with polars	3.3	9.7	0.0
Eggs with polar fusion nuclei	0.0	75.8	12.0
Embryos with developing endosperm	0.0	8.3	83.3
Degenerated embryo sacs	0.0	1.4	4.0

* Numbers of ovules examined for the respective data columns reading from left to right are: 60, 145, and 150.

Thus *Parthenium hysterophorus* resembles 36-chromosome guayule in the morphologic development of reproductive structures. Together with this guayule, it strikingly differs from the apomictic types of guayule of the polyploid series and from mariola.

SUMMARY AND CONCLUSIONS

The present study gives morphologic evidence of the occurrence of two reproductive methods in *Parthenium argentatum*—namely, sexual and apomictic. The former was characteristic of the 36-chromosome plants, and the latter of guayule with higher (polyploid) numbers of chromosomes. The *P. incanum* material also showed apomixis; that of *P. hysterophorus*, sexual reproduction.

The apomixis in *Parthenium* mainly involves generative apospory followed by unreduced pseudogamy. Since some flowers on a given plant show stages of apomictic development and others show those of the sexual reproductive cycle, apomixis in guayule is facultative.

Plants characterized by normal sexual reproduction showed an orderly sequence of events in megasporogenesis and in gametophyte development—events that succeeded each other, stage by stage, with the increase in the size of ovules. The megaspore mother cells mostly occurred in division (usually recognized as meiosis); views of tetrads of megaspores were abundant; and the uninucleate embryo sacs were associated with crushed nonfunctioning megaspores. All these features indicated that meiosis preceded embryo-sac formation. Views of fertilization of eggs, of triple fusion, and of remnants of pollen tubes in embryo sac were common in artificially pollinated flowers. Embryo and endosperm development were closely correlated. No embryos occurred in the nonpollinated material.

The morphologic features of apomictic reproduction contrasted sharply with those of sexual reproduction. Embryo-sac formation showed no orderly sequence of stages. Young stages tended to persist in fairly large ovules and overlapped, in time of occurrence, with older stages in ovules of the same size. Megaspore mother cells mostly had resting nuclei, persisted in ovules of many sizes, enlarged, became vacuolate, and assumed the characteristics of uninucleate embryo sacs. This behavior indicated omission of meiosis (apomeiosis) and formation of the embryo sac directly from the megaspore mother cell—the generative cell—without interpolation of the dyad and tetrad stages (generative apospory). Meiosis occurred but was rare, judging by the scarcity of views of megaspores, intact or degenerating. The embryo and endosperm were not closely correlated in their development. Small embryos were present in nonpollinated flowers, a fact indicating that the egg of an unreduced gametophyte may start to develop into an embryo without fertilization. Pollination, however, was highly beneficial or, perhaps, even necessary for endosperm development and for the attainment of normal embryo size. This observation agrees with the data of Powers and Rollins (1945) showing that pollination is necessary for the formation of viable seed in apomictic guayule (unreduced pseudogamy).

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The writer appreciates the coöperation of Dr. LeRoy Powers, Dr. Reed C. Rollins, and Dr. A. Dorothy Bergner, all of the Guayule Research Project of the United States Department of Agriculture, in furnishing plant material, together with relevant information. Dr. Rollins deserves special thanks for preparing material used in the pollination and fertilization studies. Dr. G. L. Stebbins, Jr., showed a constant interest in the progress of the work; his help with interpretation and analysis of certain data is thankfully acknowledged.

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PLATES

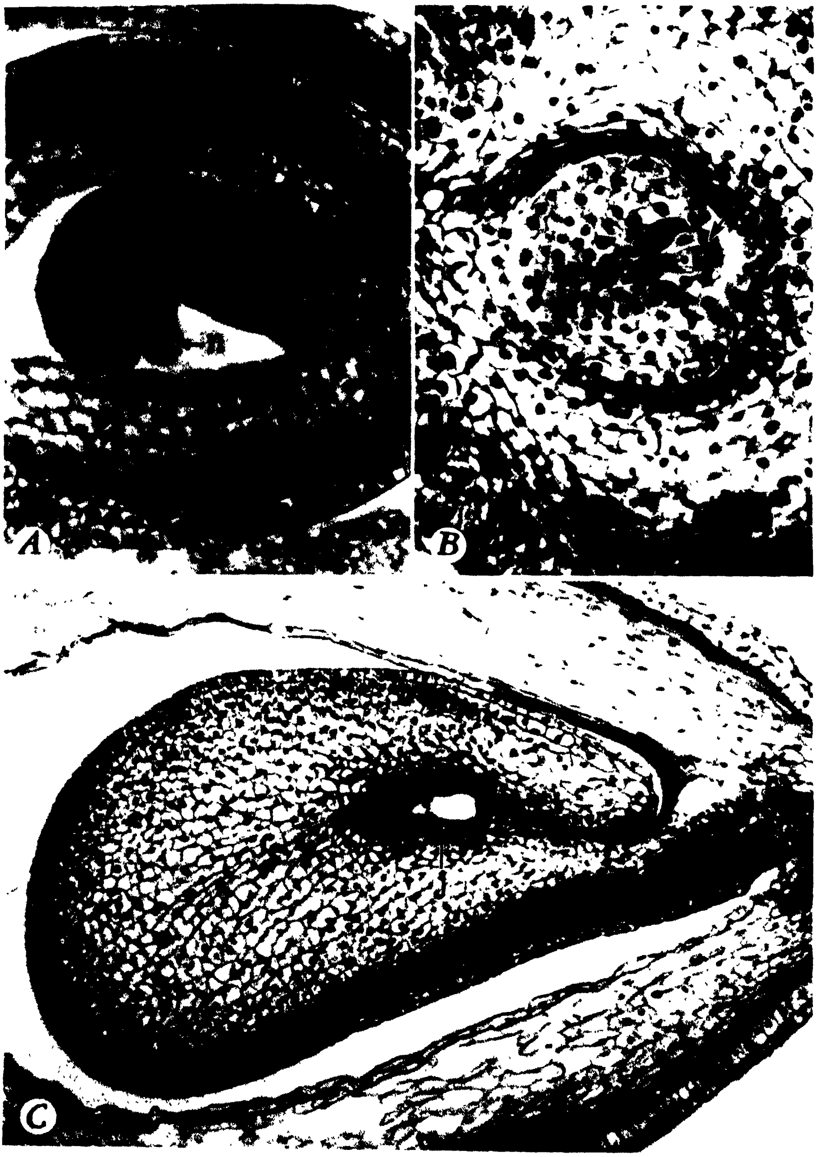

Plate. 1.—Longitudinal sections of guayule ovules (within ovaries) in different stages of development. In *A* the ovule is incompletely inverted; the nucellus (*n*) projects laterally (downward in the figure); and the integument forms a bulge above (to the left in the figure) the nucellar projection. In *B* the ovule is inverted; and the nucellar projection (*n*), partly enveloped by the integument, points downward (toward the right in the figure). The megaspore mother cell within the nucellar epidermis is in synapsis in *A* and in resting stage in *B*. The ovule in *C* contains an embryo sac in 7-celled stage, surrounded by nutritive jacket (*j*). The micropyle appears as a dark line to the right of the embryo sac. *A* was taken from a 36-chromosome plant; *B* and *C* are from a 72-chromosome type. (*A* and *B*, × 290; *C*, × 140.)

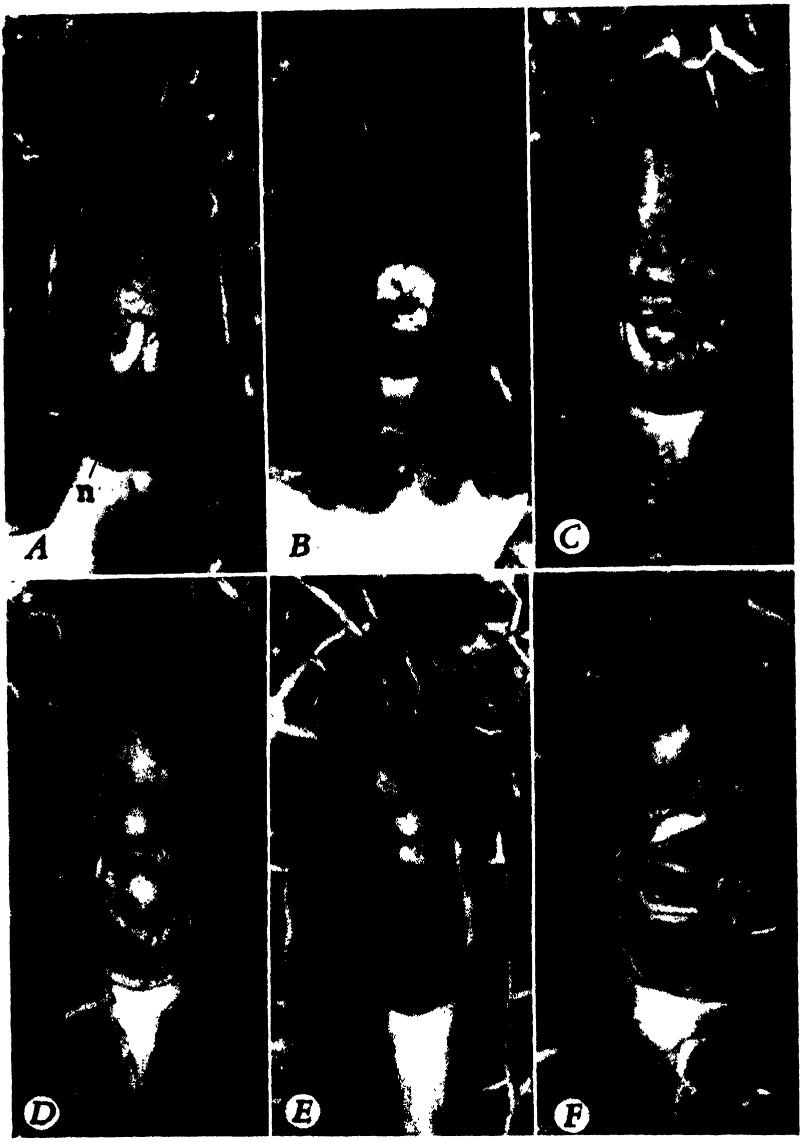

Plate 2.—Longitudinal sections of parts of nucelli and integuments from 36-chromosome guayule. The nucellar epidermis (*n*) forms a uniseriate layer around the megaspore mother cell or its products. The megaspore mother cells in *A* and *B* are in meiotic prophases. *C*, Dyad in cytokinesis. *D*, Dyad formation is complete. *E*, *F*, Tetrads of megaspores. (All × 760.)

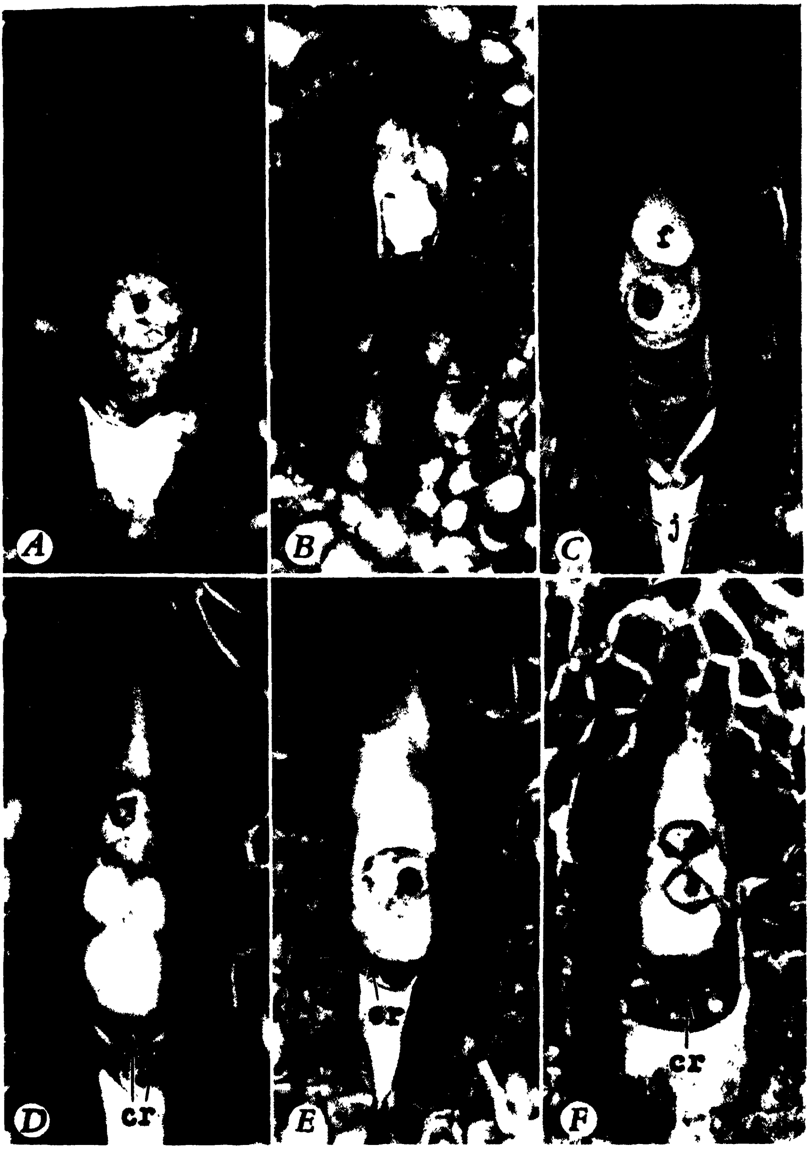

Plate 3.—*A–E*, Longitudinal sections (*B* was cut somewhat on bias) of parts of nucelli and integuments from 72-chromosome guayule. The megaspore mother cell in *A* is in early meiotic prophase; the one in *B* in diakinesis. *C*, Tetrad of megaspores. (The two megaspores nearest the micropyle were laterally adjacent, and only one of the two is visible.) The functioning megaspore appears at *f* in *C*. *D*, Uninucleate, and *E*, binucleate embryo sacs, both with crushed megaspores (*cr*). The nutritive jacket (*j*) is well defined in *C*, *D*, and *E*. *F*, Binucleate embryo sac with crushed megaspores (*cr*) from 36-chromosome guayule. (All × 760.)

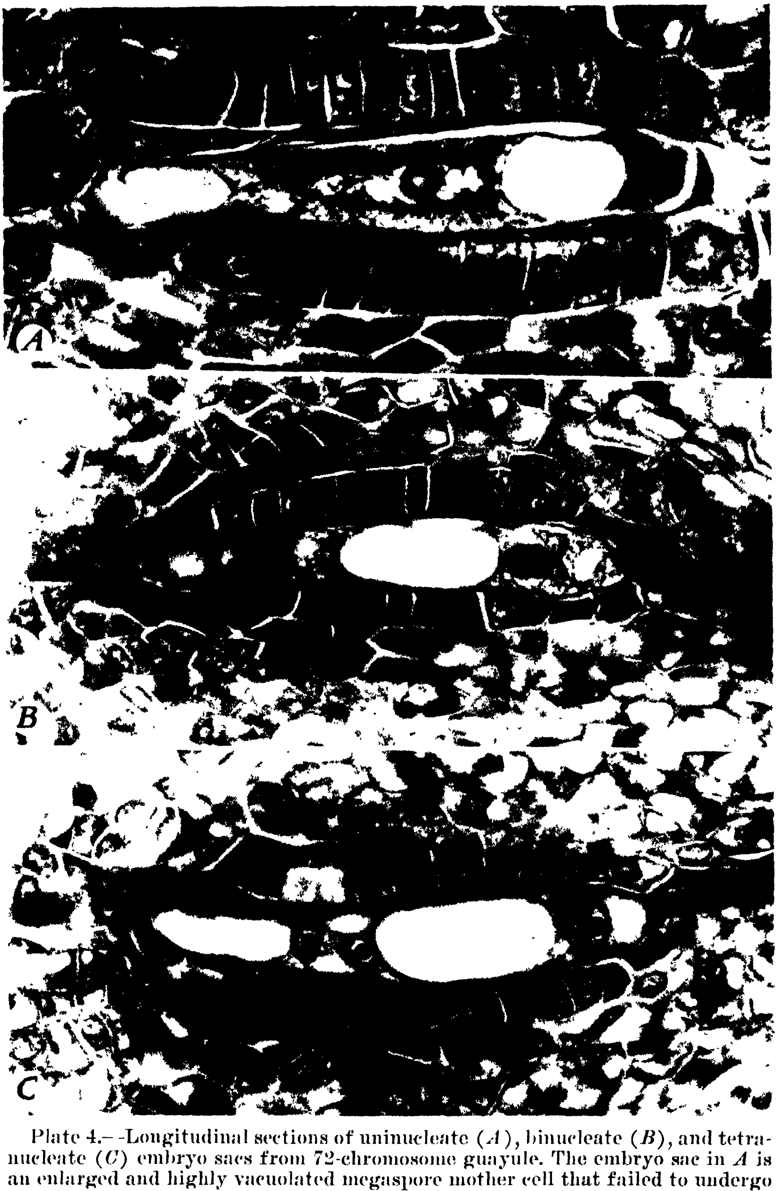

Plate 4.—Longitudinal sections of uninucleate (*A*), binucleate (*B*), and tetranucleate (*C*) embryo sacs from 72-chromosome guayule. The embryo sac in *A* is an enlarged and highly vacuolated megaspore mother cell that failed to undergo meiosis. The nucellus in *A* is partly crushed. Remnants of nucellar epidermis appear as dark streaks at the chalazal ends (to the left in the figures) of the embryo sacs in *B* and *C*. In all views the embryo sacs are bordered by uniseriate nutritive jackets of narrow cells. The embryo-sac nuclei in *B* are in division. (*A*, × 760; *B* and *C*, × 480.)

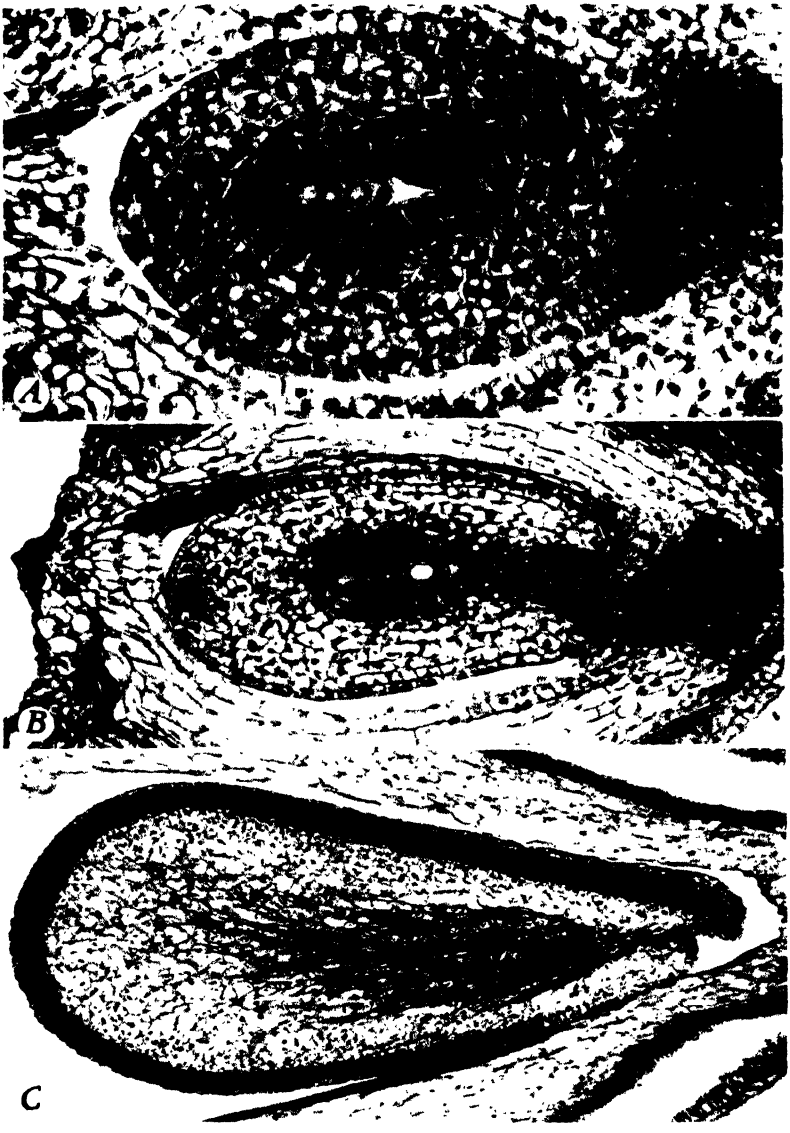

Plate 5.—Longitudinal sections of ovules (and adjacent ovarian tissues) from 36-chromosome (*A*) and polyploid (*B* and *C*) guayule. The ovule in *A* contains the dyad shown at high magnification in plate 2, *D*. The ovule in *B* contains the aposporic uninucleate embryo sac shown at high magnification in plate 4, *A*. In *C* are shown the course and extent of the ovular vascular bundle. (*A*, × 290; *B*, × 140; *C*, × 90.)

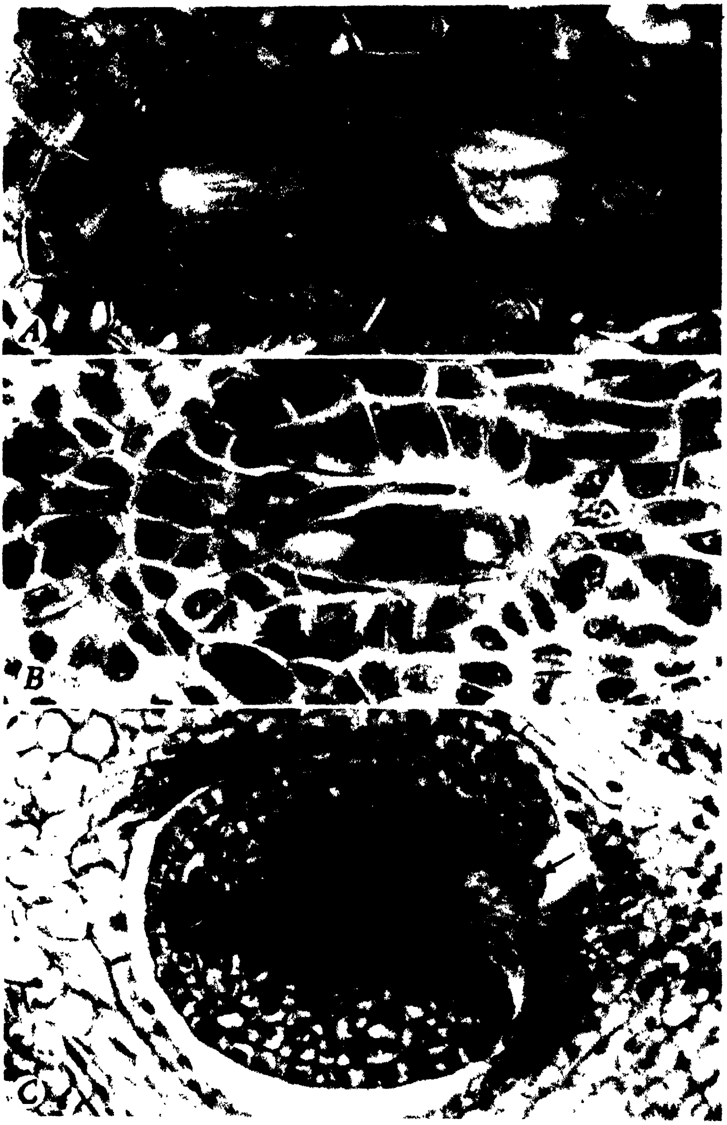

Plate 6.—*A*, An aposporic uninucleate embryo sac that has crushed and pierced the nucellar epidermis (*n*). *B*, An aposporic binucleate embryo sac, with the nucellus not yet pierced. Remnants of the spindle fibers are evident between the nuclei, but no cell wall is being formed here. *C*, Ovule (and adjacent ovarian tissues) containing an aposporic binucleate embryo sac, with one nucleus (marked with an arrow) visible outside the ovule in the ovarian cavity. All longitudinal sections from 72-chromosome guayule. (*A* and *B*, × 760; *C*, × 290.)

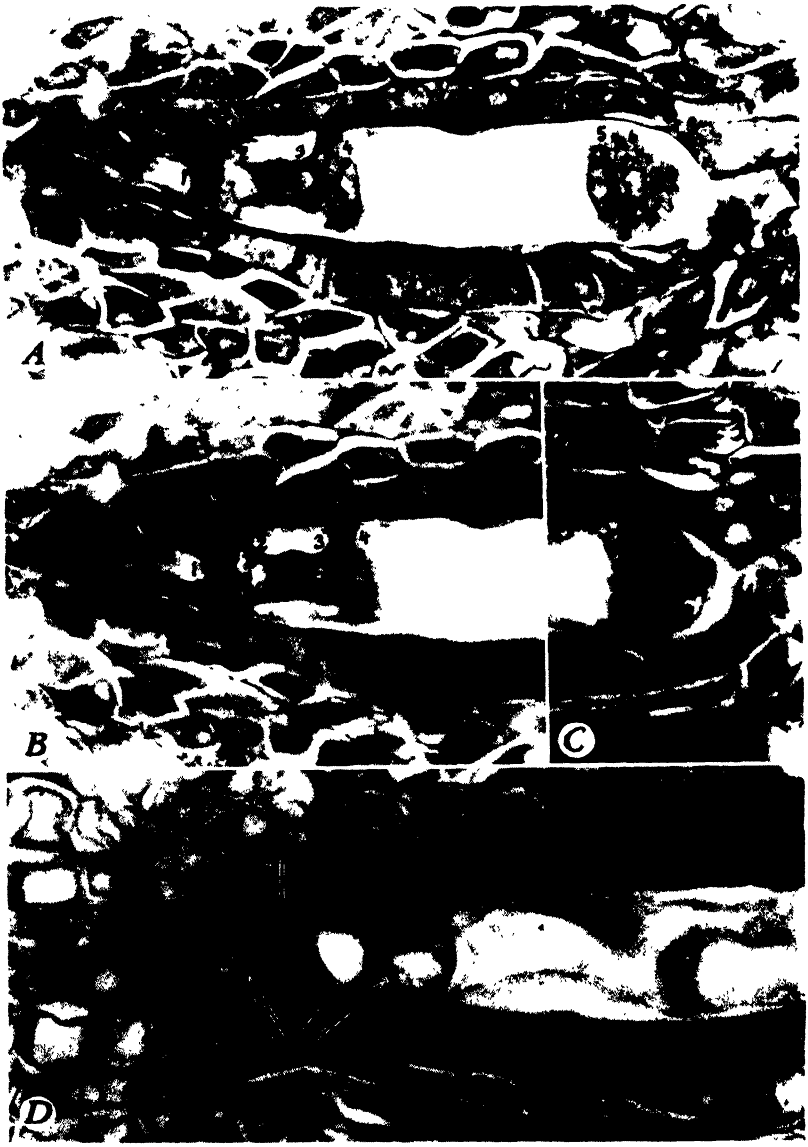

Plate 7.—A, B, and C show longitudinal sections of an 8-nucleate embryo sac from polyploid guayule. *A* and *B* present two focal planes of the same section. The section in *C* was 10 microns from that in *A*. Cytokinesis is in progress between pairs of nuclei. At 1, 2, and 3 are the antipodal nuclei; 4 and 5, the polars; 6, the egg nucleus; 7 and 8, the synergid nuclei. *D*, Longitudinal section of an embryo sac from 72-chromosome guayule, showing the antipodals (*a*). Some nucellar epidermal cells (*n*) have remained at this end of the sac. The faint outline of the egg appears to the right. (All × 760.)

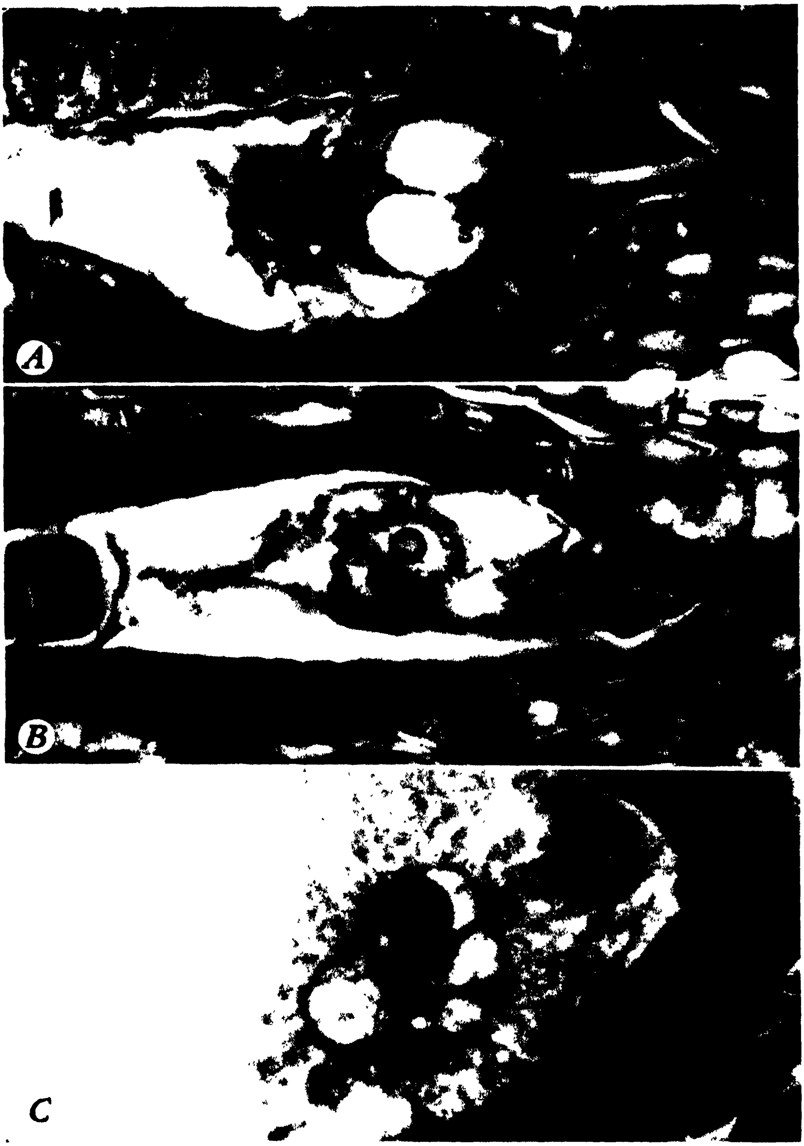

Plate 8.—Longitudinal sections of embryo sacs (and adjacent ovular layers) from 72-chromosome guayule. *A* shows two synergids (*s*) with their dense ends turned toward the micropyle and with the vacuolated ends toward the interior of the sac. A polar fusion nucleus appears to the left of the synergids. The view in *B* shows two synergids (*s*) with collapsed vacuolated ends; fusing polars to the left of the synergids; and an antipodal cell (and part of another) at the extreme left of the sac. *C*, Part of a much lobed polar fusion nucleus. (All × 760.)

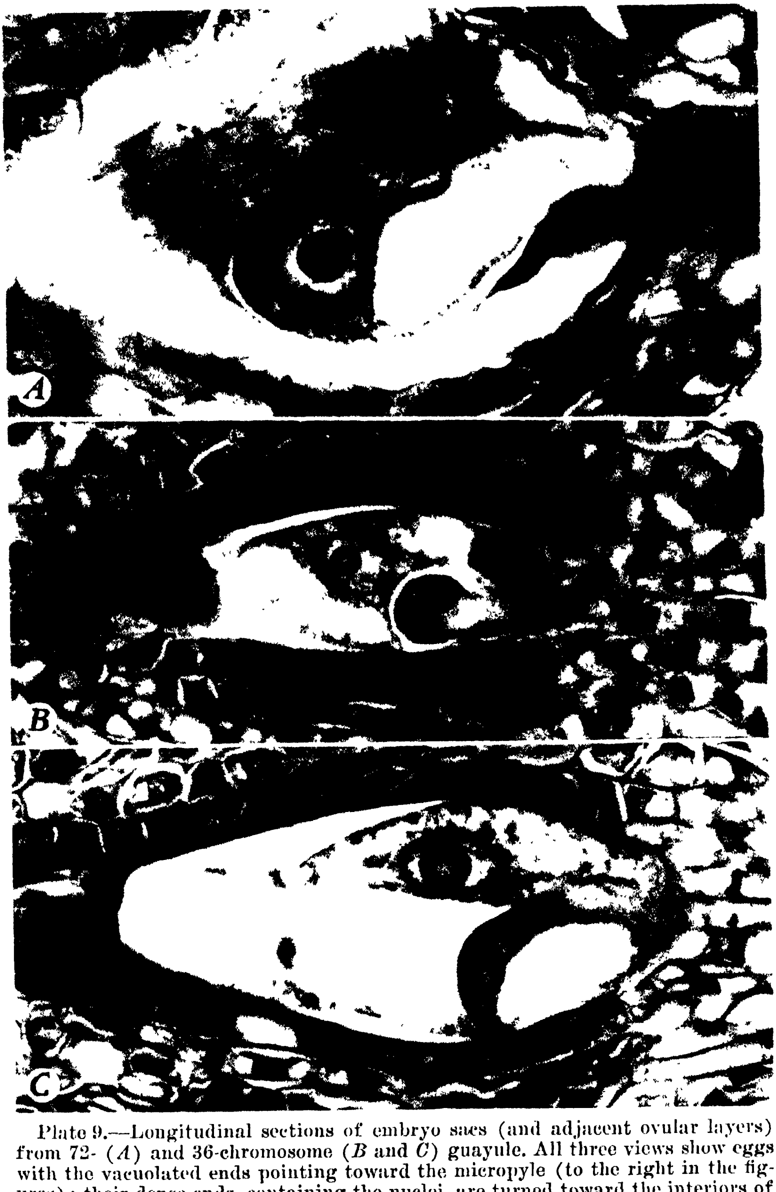

Plate 9.—Longitudinal sections of embryo sacs (and adjacent ovular layers) from 72- (*A*) and 36-chromosome (*B* and *C*) guayule. All three views show eggs with the vacuolated ends pointing toward the micropyle (to the right in the figures); their dense ends, containing the nuclei, are turned toward the interiors of the sacs. In *A*, a degenerated synergid appears to the right and below the egg; one endosperm nucleus is above the egg. (The second occurred in the next section.) Each sac in *B* and *C* contains one polar fusion nucleus to the left and above the egg. The embryo sac in *B* is younger than the one in *C*. The latter occurred in a flower that failed to become cross-pollinated and was collected long past anthesis. (All × 760.)

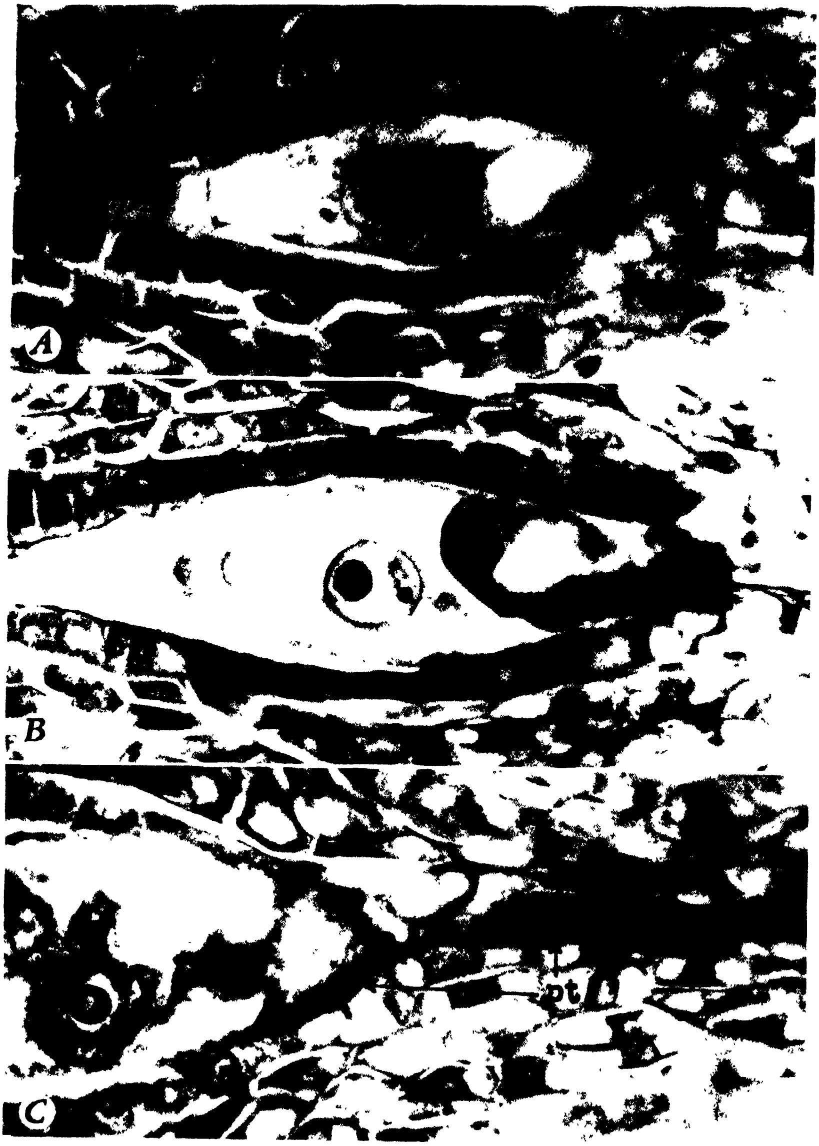

Plate 10.—*A*, *B*, Longitudinal sections of embryo sacs (and adjacent ovular layers) from 36-chromosome guayule. Darkly stained remnants of pollen tubes partly envelop the eggs in both sacs, and the small nucleoli in the primary endosperm nuclei (to the left of the eggs) indicate recent fertilization. In *A* the egg and the sperm nuclei are in process of fusion. *C*, Longitudinal section of the micropylar region of an ovule from 72-chromosome guayule, with one part of the pollen tube (*pt*) visible in the micropyle and another in the embryo sac. The egg in this sac was fertilized. The flowers used for *A* and *B* were collected 3 hours after pollination; the one used for *C*, 5 hours after pollination. (All × 760.)

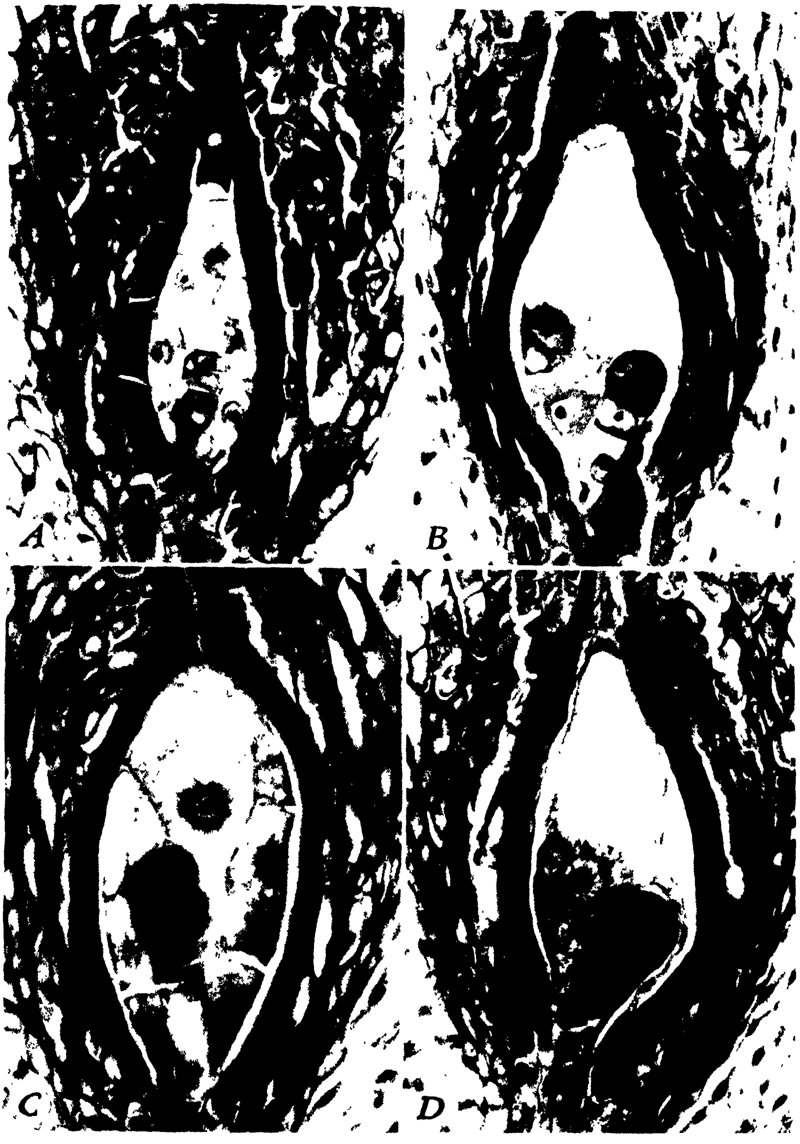

Plate 11.—Longitudinal sections of embryo sacs (and adjacent ovular layers) from 72-chromosome guayule, showing the development of embryos. The embryo sacs in *A*, *B*, and *C* contain cellular endosperm; that in *D*, a lobed polar fusion nucleus. A degenerated synergid appears at the base of the embryo in *B*. In all four sacs, the nutritive jacket is of normal thickness. The embryo sacs in *A*, *B*, and *C* occurred in cross pollinated flowers collected 3, 1, and 2 days after pollination, respectively. The flower used for *D* was selfed and was collected 3 days after pollination. (All × 290.)

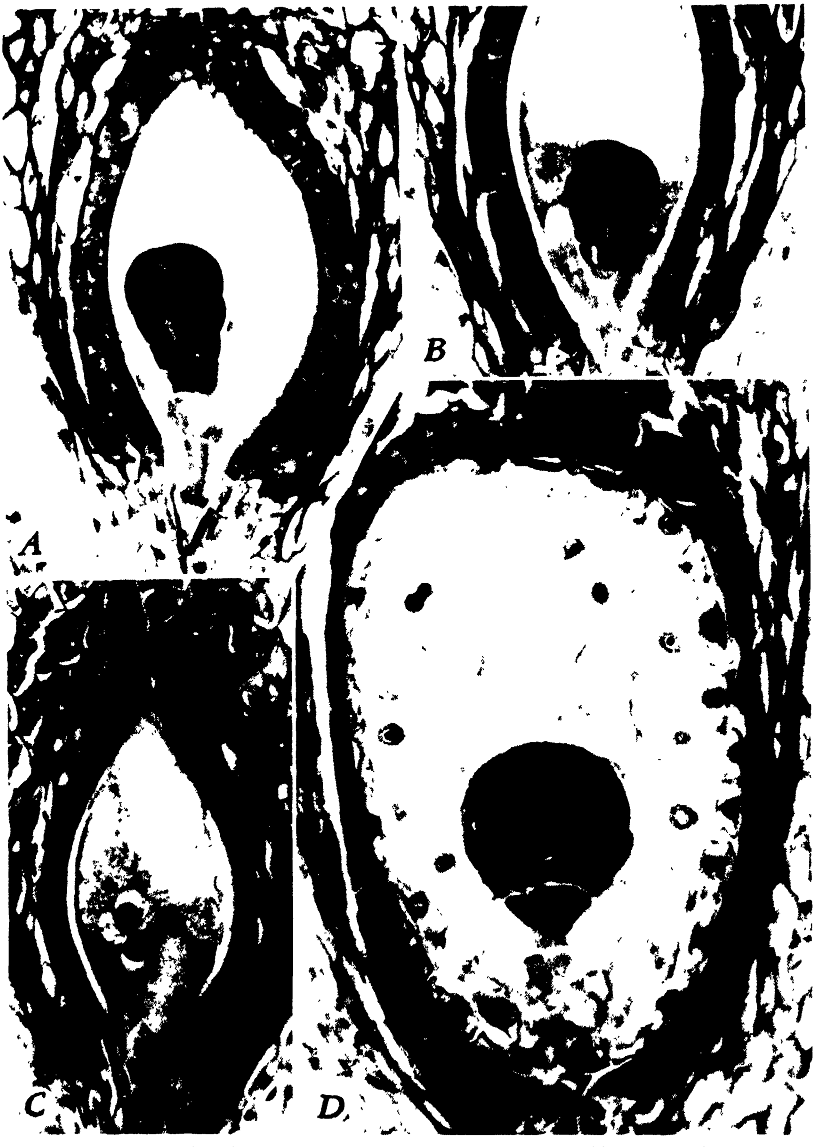

Plate 12.—Longitudinal sections of embryo sacs (and adjacent ovular layers) from 72-chromosome guayule. The embryos in *A* and *B* show the first appearance of the dermatogen. The nutritive jacket in *A* is somewhat thicker than normal. An intact synergid appears at the base and to the left of the embryo in *B*. The endosperm failed to develop in *A* and *B*. A much-lobed polar fusion nucleus is shown in *C*. The embryo in *D* is associated with cellular endosperm. The flowers used for these views were treated as follows: *A*, selfed and collected 5 days after pollination; *B* and *C*, cross-pollinated and collected 2 days after pollination; *D*, open-pollinated and collected 7 days after anthesis. (All × 290.)

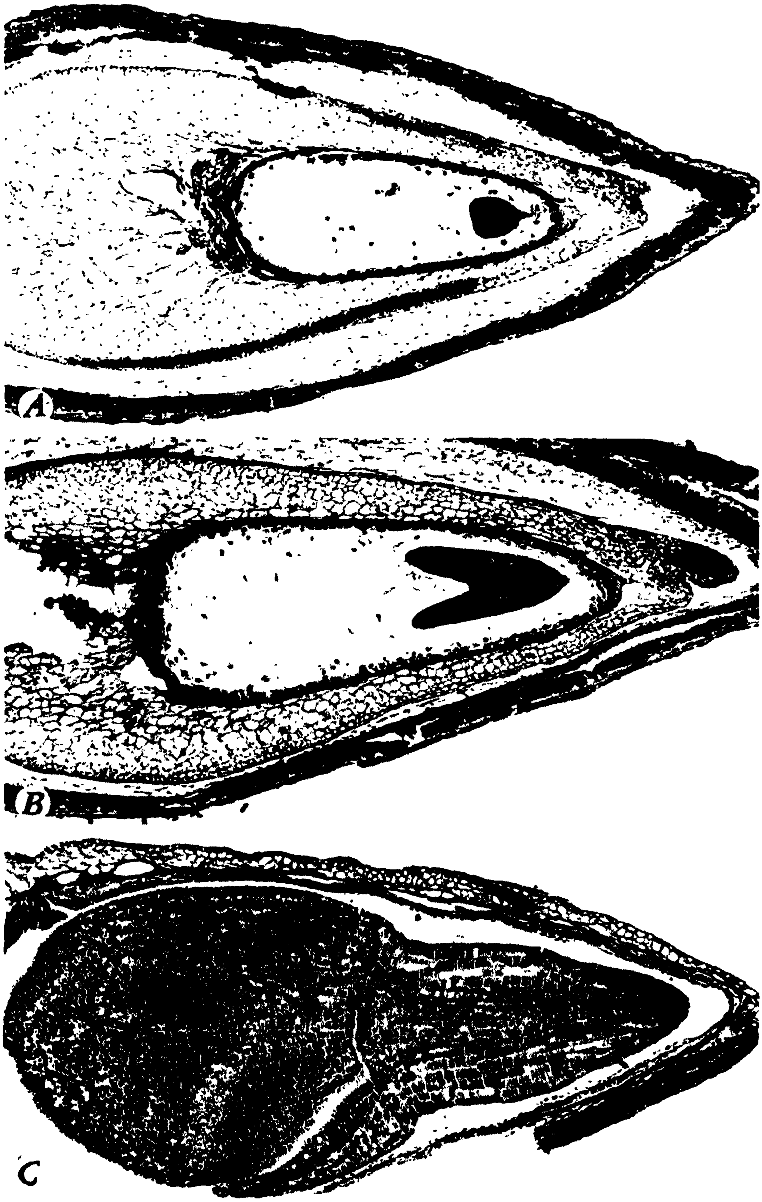

Plate 13.—Longitudinal sections of ovules (and adjacent fruit-wall layers) from 72-chromosome guayule. In *A* and *B* the ovules contain embryo sacs with cellular endosperm, and embryos showing two stages in cotyledon development. The embryo in *C* is of mature size. The flowers used in *A* and *B* were open-pollinated and were collected 5 days after pollination. The embryo in *C* is from a cross-pollinated flower collected 10 days after pollination. (All × 50.)

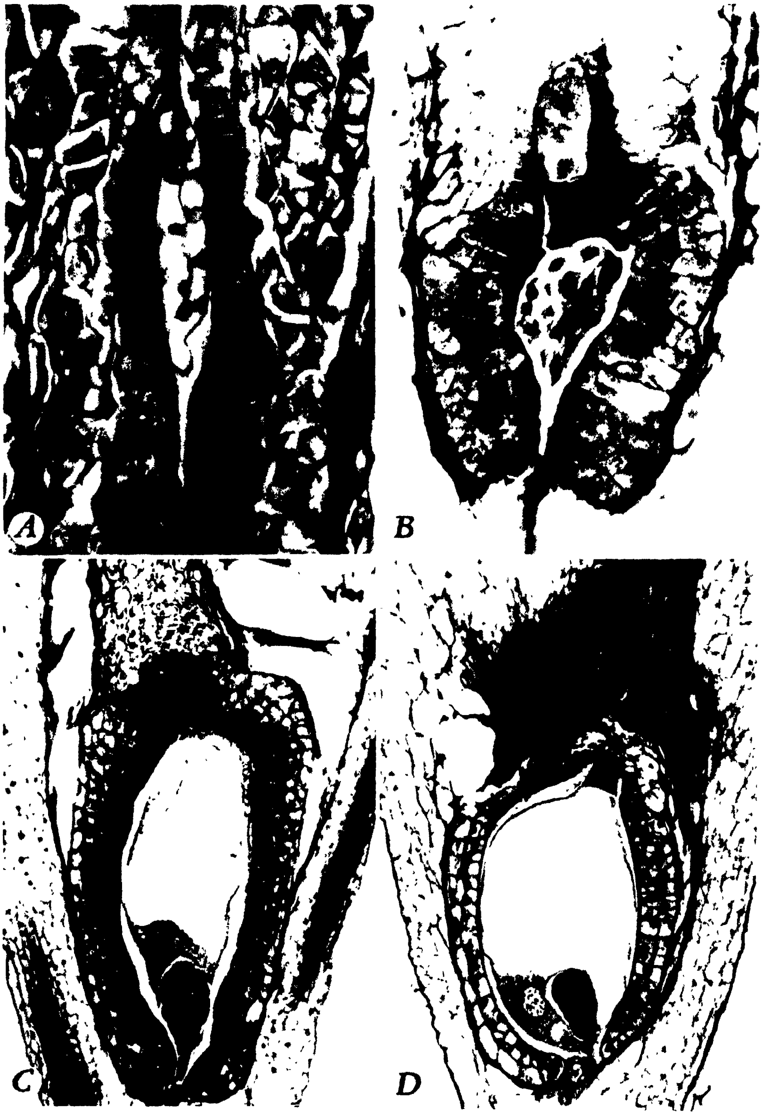

Plate 14.—Longitudinal sections of parts of ovules from 72-chromosome guayule. *A* shows in the center a collapsed nucellar epidermis between two layers of nutritive jacket. The embryo sac in *B* contains a degenerating embryo and is surrounded by a very thick multiseriate nutritive jacket. *C*, *D*, Embryo sacs from nonpollinated flowers collected 6 and 18 days after anthesis, respectively. Embryos occur in these sacs, but no endosperm. (*A*, × 480; *B*, × 290; *C* and *D*, × 140.)

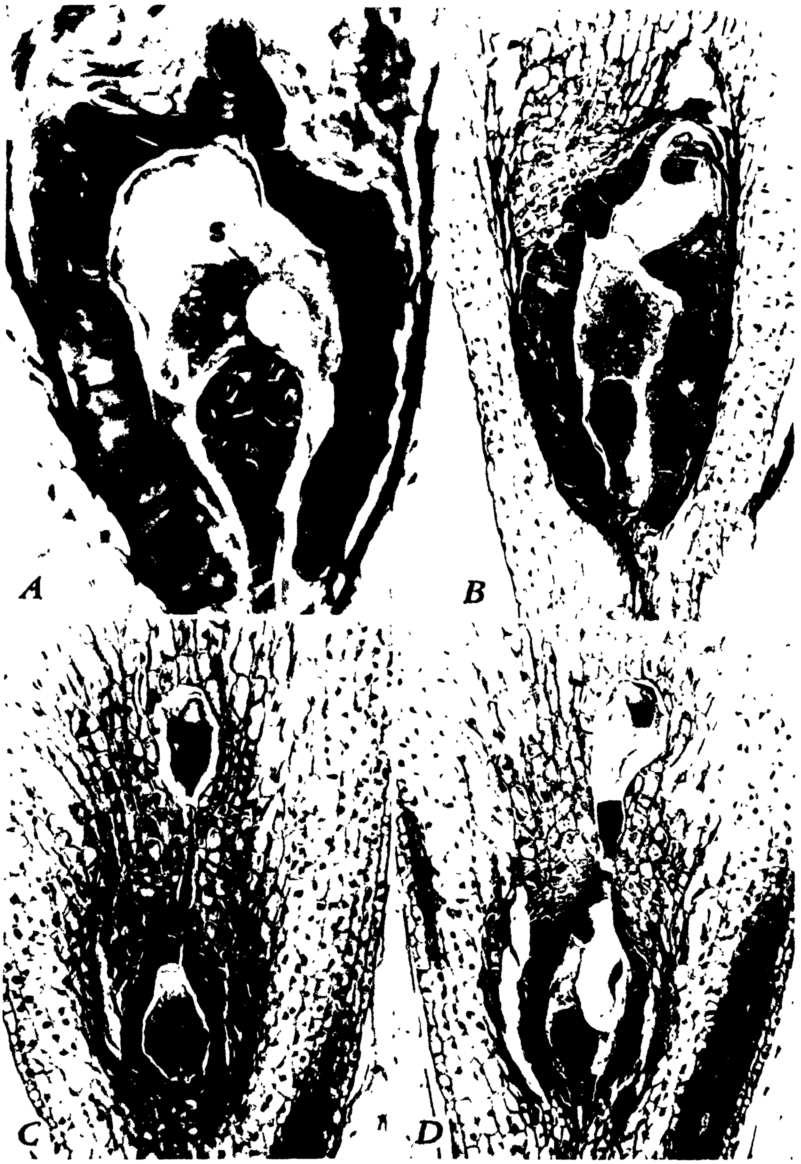

Plate 15.—Longitudinal sections of parts of ovules from 72-chromosome guayule. The embryo sac in *A* shows an embryo and a long pale synergid (*s*) that has divided into three cells. *B*, *C*, and *D* illustrate supernumerary embryo sacs that sometimes develop in addition to the sacs appearing in normal position. In *B* and *C* the extra sacs are above the ones in normal position. In *D*, two extra sacs are present, one in the cavity of the sac in normal position, the other above it. An embryo (*B*) and eggs (*C* and *D*) occur in the extra sacs. (*A*, × 290; *B–D*, × 140.)

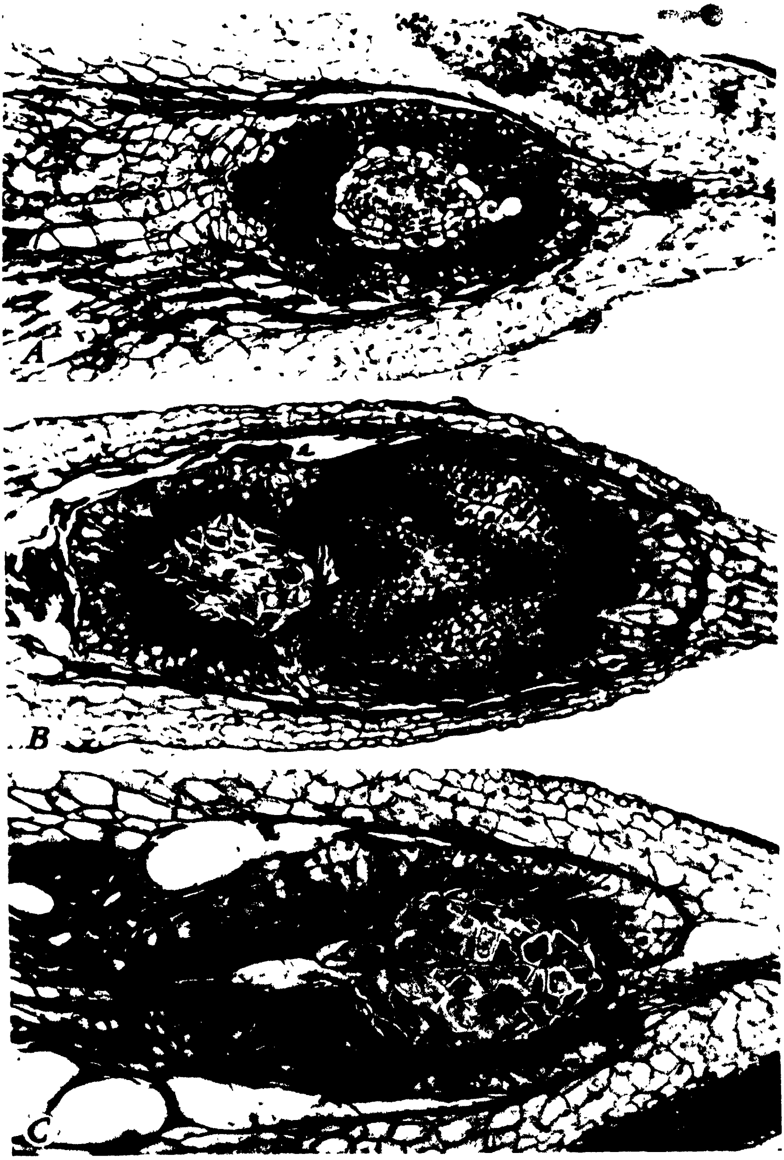

Plate 16.—Longitudinal sections of degenerating embryo sacs and ovular tissues enclosing them, from apomictic guayule. In *A* the embryo is imbedded in the thick nutritive jacket and is associated with no endosperm. In *B* the embryo is of abnormal shape, some endosperm occurs to the left of the embryo, and the nutritive jacket is very thick. *C*, Embryo sac without an embryo, but with some degenerating endosperm and a thick nutritive jacket. (All × 140.)

HILGARDIA

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LONGEVITY, OR LIFE HISTORIES, OF LEAFHOPPER SPECIES ON VIRUS-INFECTED AND ON HEALTHY PLANTS[1]

HENRY H. P. SEVERIN[2]

INTRODUCTION

IN DISCUSSING the effect of the virus on the insect vector, Leach (1940)[3] has suggested that the question of whether a virus-infected plant is a more favorable host plant than a healthy plant, has been given little attention in virus studies.

Carter (1939), working with yellow-spot of pineapple, found that infected weeds of *Emilia sonchifolia* DC maintain, on an average, higher populations of onion thrips, *Thrips tabaci* Lind., than do healthy plants. He concluded that diseased plants may persist for a longer time, with a mass of curled leaves affording satisfactory shelter for the vector, than do healthy plants which grow rapidly, mature, and die.

It is a well-known fact among entomologists who have carried on field investigations with the beet leafhopper, *Eutettix tenellus* (Baker), that when sugar beets are harvested, the adults fly to other food plants, and a high mortality occurs with the change in host plants. During the 1925 outbreak of the pest, and after the beet tops became dry, enormous numbers of leafhoppers were found in bean fields (Severin and Henderson, 1928), and dead adults were commonly found with their mouth parts inserted in the bean leaves. Tomato plants are favorable host plants of the curly-top virus, but are unfavorable food plants of the beet leafhopper. The decrease in longevity of the males and females on tomato plants has been discussed in a previous paper (Severin, 1928).

During the past seventeen years, 9 leafhopper species have been reported by the author (Severin, 1929, 1934, 1945) to be capable of transmitting the California aster-yellows virus. During the course of an investigation on the longevity of noninfective short-winged *Macrosteles divisus* (Uhler) and long-winged aster leafhoppers—a biological race of the same species (Severin,

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[1] Received for publication November 15, 1945.

[2] Entomologist in the Experiment Station.

[3] See "Literature Cited" at the end of the paper for complete citations, referred to in the text by author and date of publication.

1940) which had completed their nymphal stages on mildew-resistant Sacramento barley nonsusceptible to the California aster yellows—it was found that low populations of adults of the short-winged leafhoppers were reared, whereas, the long-winged died on healthy celery after the first molt. It was also demonstrated that when both vectors were allowed to reproduce on celery infected with the virus, high populations of adult short-winged aster leafhoppers and low populations of adult long-winged forms were obtained.

Among a large number of newly discovered leafhopper vectors, some species reproduced on both healthy and infected celery; other species multiplied on diseased celery and asters only, and died when transferred to healthy celery and asters; and still other species lived long enough to complete the latent period of the virus but failed to reproduce, and also died when transferred to healthy celery or asters.

Kunkel (1926) found that the latent period of the New York aster-yellows virus in the short-winged aster leafhoppers, using 30 to 100 nymphs or adults in many tests, varied between 10 to 19 days at 70° F. Kunkel (1932) also reported that the latent period of the California aster-yellows virus in the short-winged aster leafhopper, using 30 to 125 nymphs, varied from 19 to 26 days, with an average of 23 days. The latent period of the virus in *Texananus lathropi* Baker varied from 7 to 33 days, in *T. latipex* DeLong from 8 to 37 days, and in *T. spatulatus* Van Duzee from 6 to 35 days, as determined by Severin (1945). Any species of leafhopper which failed to survive on diseased celery or asters for at least 1 week could not be tested as a possible vector, since the latent period of the virus was not completed in the insects.

The present paper deals with the longevity of 9 species of leafhoppers on virus-infected and healthy plants, 5 of which have been previously reported as vectors of the virus (Severin, 1929, 1934, 1945). Another aspect of the problem was to compare the total duration of the nymphal stages of 2 species of phlepsid leafhoppers on healthy and diseased celery plants, and of the beet leafhopper on healthy and curly-top sugar beets.

METHODS

The production of noninfective short-winged and long-winged aster leafhoppers has been described in previous papers (Severin 1929, 1942). The method of obtaining noninfective *Texananus lathropi, T. latipex,* and *Cloanthanus irroratus* (Van Duzee) was similar to that reported for the beet leafhopper (Severin, 1921). The technique used in obtaining noninfective populations of *T. spatulatus* (Van Duzee) has been described in a previous paper (Severin, 1945). In determining the longevity of species of leafhoppers, recently molted adults were used, reared from high populations of last instar nymphs.

LONGEVITY OF SPECIES OF LEAFHOPPERS ON VIRUS-INFECTED AND ON HEALTHY PLANTS

TEXANANUS DENTICULUS OSBORN AND LATHROP

This leafhopper (plate 1, *G, H*) completed the nymphal stages on infected celery, but died on healthy, control celery plants. Fifty males and 50 females were kept singly, each on a healthy celery plant, and a daily record was taken of the mortality of the adults. The longevity of the males ranged from 2 to 20 days, with an average of only 6.9 days; females, from 2 to 33 days, with an average of only 8.4 days.

TEXANANUS PERGRADUS DELONG

This leafhopper vector (Severin, 1945) completed the nymphal stages on experimentally and naturally infected celery, but when transferred to healthy celery the adults died. In testing the efficiency of this vector in transmitting the virus, 50 males and 50 females were kept singly, each on a healthy celery plant during adult life. The longevity of the males ranged from 3 to 8 days, with an average of only 4.8 days; females, from 2 to 12 days, with an average of only 6.7 days.

In another test, 2 lots of 10 recently molted, infective males and females were kept singly, each on a healthy celery plant, and the same number of adults were kept singly on diseased celery plants. On healthy celery, the longevity of 10 males ranged from 3 to 9 days, with an average of only 6.1 days; females, 3 to 22 days, with an average of only 11.6 days.

On infected celery, 10 males and 10 females were alive at the end of 24 days; then equal numbers of males and females were combined on experimentally and naturally infected celery and high populations of adults were reared on both.

TEXANANUS SPATULATUS VAN DUZEE

A comparison was made of the longevity of each leafhopper which transmitted the virus to celery (Severin, 1945) with each adult which failed and, hence, was feeding continuously on healthy celery. The longevity of 34 males which induced the disease ranged from 4 to 144 days, with an average of 45.6 days; and of the 29 females which caused infections, 4 to 198 days, with an average of 51.3 days. The average adult life of 66 males which failed to transmit the virus was 17.9 days; and of 71 females, 20.3 days. Evidently a virus-infected plant prolonged the life of this leafhopper even before symptoms of the disease developed.

This leafhopper completed the nymphal stages on diseased asters. Each of 50 recently molted males and 50 females, which completed the nymphal stages on infected asters, was transferred to a healthy aster, and a daily record of the mortality was taken. The males lived from 1 to 23 days, with an average of only 3.1 days; females, 1 to 28 days, with an average of only 6.3 days. The life cycle was not completed on healthy asters.

CLOANTHANUS IRRORATUS (VAN DUZEE)

The life cycle of this leafhopper was completed on diseased asters. The longevity of 50 males, kept singly, each on a healthy aster, ranged from 1 to 11 days, with an average of only 4.1 days; and of 50 females from 2 to 25 days, with an average of only 10.2 days. The leafhoppers did not complete their life history on healthy asters.

EUSCELES MACULIPENIS DeLONG

The nymphal stages of this leafhopper were completed on diseased asters. The longevity of 50 males on healthy asters ranged from 1 to 35 days, with an average of only 3.4 days; 50 females, 1 to 24 days, with an average of only 3.5 days. This leafhopper also failed to complete the life cycle on healthy asters.

GEMINATE LEAFHOPPER, COLLADONUS GEMINATUS (VAN DUZEE)

As reported in a previous paper (Severin, 1934), the geminate leafhopper was collected on large asters under natural conditions, but a high mortality occurred on small asters in the greenhouse. It was found that adults which were fed on small healthy asters died within a week. The longevity of the last surviving adult of 11 lots of 50 recently molted adults on large healthy asters, ranged from 26 to 123 days, with an average of 84.5 days. Sometimes nymphs completed the nymphal stages when fed on large healthy asters. Nymphs reached the adult stage on diseased asters.

MOUNTAIN LEAFHOPPER, COLLADONUS MONTANUS (VAN DUZEE)

The nymphal stages of the mountain leafhopper were completed on infected asters. The adult life on small asters ranged from 2 to 15 days, with an average of only 4.3 days.

IDIODONUS KIRKALDYI (BALL)

There are numerous leafhopper species which live on diseased celery and asters, but when transferred to healthy plants they die. One illustration will suffice. No attempt has been made to rear *Idiodonus kirkaldyi* on coast sagebrush, *Artemesia california*, its natural host plant. One lot of 4 adults was kept on infected celery for 66 days, whereas the longevity of 10 males on healthy celery ranged from 1 to 10 days, with an average of only 3.2 days; females, 1 to 6 days, with an average of only 3.4 days. On healthy asters the adult life of the males ranged from 1 to 10 days, with an average of only 8.6 days; females, 1 to 32 days, with an average of only 7.8 days.

COMPARISON OF DURATION OF NYMPHAL STAGES ON HEALTHY AND DISEASED PLANTS

TEXANANUS LATHROPI BAKER

A comparison was made of the interval or period between molts (stages, or stadia—not to be confused with longevity of adults) and the total duration of the nymphal stages of *Texananus lathropi* (plate 1, *A, B, C, D, E, F, I*) reared singly on healthy and diseased celery plants (table 1). In the first experiment, the average total duration of the nymphal stages of the males was 69 days on healthy celery and 51.6, or 7.4 days less, on infected celery; and of the females, 63 days on healthy celery and 60.9, or 2.1 days less, on diseased celery.

In the second experiment, 8 males reared on healthy celery required an average of 51.7 days and 1 male, 52 days on infected celery, or 0.3 days longer on healthy celery.

TABLE 1

DURATION OF STADIA *Texananus lathropi* ON HEALTHY AND ON INFECTED CELERY PLANTS
(Expressed as number of days between molts)

	First instar	Second instar	Third instar	Fourth instar	Fifth instar	Sixth instar	Total
Four males hatched on May 1, and reared on healthy celery plants (first experiment)							
	11	10	9	14	18	.	62
	12	10	9	16	20	..	67
	14	10	10	26	8	..	68
	14	23	13	12	16	1	79
Average	*12.7*	*13.2*	*10.2*	*17.0*	*15.5*	*1*	*69.0*
Five males hatched on May 1, and reared on infected celery plants (first experiment)							
	12	5	9	9	12	..	47
	12	4	8	10	14	..	48
	12	5	8	9	14	..	48
	11	6	7	9	15	..	48
	12	10	9	16	20	..	67
Average	*12.0*	*6.0*	*8.2*	*10.6*	*15.0*	..	*51.6*
Six females hatched on May 1, and reared on healthy celery plants (first experiment)							
	10	7	7	8	16	..	48
	11	8	8	13	16	..	56
	11	8	7	11	20	..	57
	10	7	6	19	21	.	63
	12	11	14	17	17	.	71
	15	23	14	13	18	..	83
Average	*11.5*	*10.7*	*9.3*	*13.5*	*18.0*	.	*63.0*
Seven females hatched on May 1, and reared on infected celery plants (first experiment)							
	13	4	7	4	24	..	52
	12	7	9	10	16	..	54
	12	7	10	9	17	..	55
	15	11	6	16	16	..	64
	13	12	10	10	21	..	66
	14	13	10	10	20	..	67
	15	11	13	10	19	..	68
Average	*13.4*	*9.3*	*9.3*	*9.9*	*19.0*	..	*60.9*
Eight males hatched on August 30, and reared on healthy celery plants (second experiment)							
	12	7	5	8	13	..	45
	10	7	6	9	14	..	46
	11	8	6	8	15	..	48
	12	7	5	8	17	..	49
	11	8	7	9	15	..	50
	11	8	8	10	18	..	55
	12	8	9	8	19	..	56
	12	9	10	11	9	14	65
Average	*11.4*	*7.7*	*7.0*	*8.9*	*15.0*	*14*	*51.7*

TABLE 1 (*Continued*)

	First instar	Second instar	Third instar	Fourth instar	Fifth instar	Sixth instar	Total
One male hatched on August 30, and reared on infected celery plant (second experiment)							
	12	9	7	6	18	..	52
Three females hatched on August 30, and reared on healthy celery plants (third experiment)							
	12	7	5	9	18	..	51
	12	8	7	9	19	..	55
	12	11	11	12	23	..	69
Average................	*12.0*	*8.7*	*7.7*	*10.0*	*20.0*	..	*58.3*
Eleven females hatched on August 30, and reared on infected celery plants (third experiment)							
	10	8	6	9	14	..	47
	11	7	6	9	15	..	48
	10	7	6	9	16	..	48
	12	6	5	3	16	..	42
	10	8	6	10	15	..	49
	12	7	8	8	15	..	50
	10	7	7	10	17	..	51
	12	7	8	7	17	..	51
	13	8	7	11	10	5	54
	11	8	6	10	20	..	55
	11	7	6	11	24	..	59
Average................	*11.1*	*7.3*	*6.5*	*8.8*	*16.3*	*5*	*50.4*

In the third experiment, the average total duration of the nymphal stages of the females was 58.3 days on healthy celery and 50.4, or 7.9 days less, on diseased celery.

Two nymphs reared on healthy celery passed through six molts; all others, whether on healthy or diseased plants, molted five times.

The life history of *Texananus lathropi* was repeated, but only the total duration of the nymphal stages was determined on healthy and diseased celery plants. The average total duration of the nymphal stages on healthy versus diseased celery plants (table 2) for males was 40.7 days as compared with 38.6 days, and for females, 46.0 days as compared with 40.2 days, demonstrating that the average total duration of the nymphal stages is less on diseased plants.

A statistical analysis of the data on the duration of the nymphal stages of each adult reared on healthy and diseased plants showed no significance.

A high mortality of nymphs occurred when reared on healthy celery plants. A nymphal stage may be greatly prolonged, sometimes a month or longer, but such nymphs die before molting again. Sometimes nymphs die in the process of molting. The mortality of the nymphs was determined with 80 nymphs which hatched on 3 successive days and were placed on 3 healthy celery plants. The nymphs were counted 38 days after hatching and 56 were alive. Forty-four, or 55.0 per cent, of the nymphs reached the adult stage.

A comparison was made of the number of adults reared on healthy and infected celery plants. After their last molt, 10 males and 10 females were confined, 1 pair in each cage which contained either a healthy or diseased celery plant. The number of adults reared from each pair was as follows: healthy celery, 51, 63, 64, 113, and 158, or an average of 97; infected celery, 229, 231, 345, 369, and 395, or an average of 341.

TABLE 2

TOTAL DURATION OF NYMPHAL STAGES OF *Texananus lathropi* HATCHED ON APRIL 26, REARED ON HEALTHY AND ON INFECTED CELERY PLANTS

Number of noninfective males reared	Total duration of nymphal stages of males, days	Number of noninfective females reared	Total duration of nymphal stages of females, days
Reared on healthy celery plants			
1	34	0	..
0	..	1	35
2	39	1	39
2	40	1	40
3	41	0	..
1	42	0	..
0	..	1	43
1	44	2	44
2	45	0	..
0	..	2	46
0	..	1	47
0	..	1	49
0	..	2	50
0	..	1	52
0	..	1	55
0	..	1	56
0	..	1	62
Total 12	*Average 40.7*	*Total 16*	*Average 46.0*
Reared on infected celery plants			
2	34	2	34
0	.	2	36
1	38	1	38
1	39	3	39
1	40	0	..
0		1	41
1	42	1	42
0	..	3	45
0	..	2	47
Total 6	*Average 38.6*	*Total 15*	*Average 40.2*

TEXANANUS SPATULATUS VAN DUZEE

A comparison was made of the duration of periods between molts of *Texananus spatulatus* reared on healthy celery with those of leafhoppers on celery infected with aster yellows; and the same comparison was made with rearing on healthy sugar beets and on curly-top beets (table 3), although this leafhopper is not a vector of the curly-top virus.

The average total duration of the nymphal stages of the males was 112

TABLE 3

DURATION OF STADIA OF *Texananus spatulatus* REARED ON CELERY INFECTED WITH ASTER YELLOWS, ON SUGAR BEETS INFECTED WITH CURLY TOP, AND ON HEALTHY PLANTS

Date hatched	Duration of stadia, days						
	First instar	Second instar	Third instar	Fourth instar	Fifth instar	Sixth instar	Total
			Males reared on healthy celery plants				
September 30.	12	7	5	9	19	..	52
September 25	10	10	14	19	33	..	86
September 25...	10	10	8	45	14	..	87
September 24.	10	11	8	43	16	..	88
September 24	10	11	10	58	33	..	112
September 2..	11	7	6	11	62	18	115
August 30...	30	13	9	59	21	..	132
Average	*13.3*	*9.9*	*8.6*	*34.7*	*26.9*	*18.0*	*112*
			Males reared on infected celery plants				
September 25	6	14	9	38	17	..	84
September 25	10	10	17	53	21	..	111
September 24	9	12	11	55	26	..	113
Average	*8.3*	*12.0*	*12.3*	*48.7*	*21.3*	..	*102.7*
			Females reared on healthy celery plants				
September 25	10	10	11	55	16	..	102
September 25	12	9	29	44	18	..	112
Average	*11.0*	*9.5*	*20.0*	*49.5*	*17.0*	..	*107*
			Females reared on infected celery plants				
September 25	9	11	10	46	17	..	93
September 24	11	11	15	49	14	..	100
September 26	11	11	11	42	30	..	105
September 28	6	11	14	65	18	..	114
September 26	11	10	14	61	19	..	115
Average	*9.6*	*10.8*	*12.8*	*52.6*	*19.6*	..	*105.4*
			Male reared on healthy sugar beet				
September 25	9	12	12	59	19	..	111
			Males reared on infected sugar beets				
September 25	9	11	9	47	15	..	91
September 25	9	11	11	48	17	..	96
Average	*9.0*	*11.0*	*10.0*	*47.5*	*16.0*	..	*93.5*

TABLE 3 (*Continued*)

Date hatched	Duration of stadia, days						
	First instar	Second instar	Third instar	Fourth instar	Fifth instar	Sixth instar	Total
Female reared on healthy sugar beet							
September 25	9	10	13	52	8	19	111
Females reared on infected sugar beets							
September 25	9	11	10	38	17	..	85
September 25	9	11	11	40	15	..	86
September 25	9	11	11	47	17	..	95
September 25	9	11	14	62	16	..	112
Average	*9.0*	*11.0*	*11.5*	*45.4*	*16.2*	..	*94.5*

days on healthy celery and 102.7, or 9.3 days less, on infected celery; and of the females, 107 days on healthy celery and 105.4, or 1.6 days less, on diseased celery.

The total duration of the nymphal stages on healthy versus curly-top beets (table 3) for 1 male was 111 days as compared with an average of 93.5 days for 2 males, and for 1 female, 111 days, compared with an average of 94.5 days for 4 females. These results demonstrate that the average total duration of the nymphal stages is less on diseased plants, even though only 8 adults were reared.

Two nymphs passed through six molts, all others through five.

A statistical analysis of the data on the duration of the nymphal stages of each adult reared on healthy and diseased plants showed no significance.

BEET LEAFHOPPER, EUTETTIX TENELLUS (BAKER)

A comparison was made of the total duration of the nymphal stages of the beet leafhopper, *Eutettix tenellus,* reared on healthy and on curly-top sugar beets (table 4). In three experiments the males required 4.4, 10.3, and 1 days longer on curly-top than on healthy beets; and the females, 1.1, 1.6, and 10.2 days longer. There was no evidence to show that the total duration of the nymphal stages is shorter on curly-top beets than on healthy ones.

SUMMARY

Evidence is presented in this paper that 9 species of leafhopper vectors tested completed the nymphal stages on celery or asters infected with the California aster-yellows virus, but that the adults died when transferred to healthy celery or to asters. The longevity of single males and females of *Texananus spatulatus* was longer on infected celery before symptoms developed than on healthy celery.

The average total duration of the nymphal stages of *Texananus lathropi* was less on infected than on healthy celery, with one exception: where small num-

TABLE 4

TOTAL DURATION OF NYMPHAL STAGES OF *Eutettix tenellus* REARED ON HEALTHY AND ON CURLY-TOP SUGAR BEETS

Number of males reared	Total duration of nymphal stages of males, days	Number of females reared	Total duration of nymphal stages of females, days
Hatched April 30, and reared on healthy sugar beets (first experiment)			
8	36	6	36
4	37	7	37
2	38	4	38
7	39	6	39
6	40	4	40
3	41	6	41
1	42	3	42
1	43	2	43
1	44	1	44
0	..	1	45
1	46	0	..
0	..	1	49
0	..	1	51
Total 34	*Average 40.6*	*Total 42*	*Average 42.1*
Hatched April 30, and reared on curly-top sugar beets (first experiment)			
1	37	0	37
0	38	1	38
1	39	0	39
1	40	1	40
2	41	3	41
6	42	6	42
3	43	4	43
1	44	4	44
1	45	1	45
3	48	1	48
2	49	1	49
1	52	0	..
1	54	0	..
0	..	1	52
1	58	0	..
Total 24	*Average 45.0*	*Total 23*	*Average 43.2*
Hatched May 1, and reared on healthy sugar beets (second experiment)			
3	35	2	35
17	36	23	36
1	37	3	37
3	38	1	38
2	39	0	..
0	..	4	40
0	..	2	41
0	..	1	43
Total 26	*Average 37.0*	*Total 36*	*38.6*

TABLE 4 (*Continued*)

Number of males reared	Total duration of nymphal stages of males, days	Number of females reared	Total duration of nymphal stages of females, days
Hatched May 1, and reared on curly-top sugar beets (second experiment)			
2	39	0	.
3	40	3	40
6	41	7	41
14	42	6	42
7	43	16	43
9	44	5	44
4	45	5	45
5	46	3	46
6	47	5	47
2	48	8	48
1	49	0	..
2	51	1	49
1	52	1	.
1	53	0	.
1	69	0	..
Total 64	*Average 47.3*	*Total 60*	*Average 40.2*
Hatched May 3, and reared on healthy sugar beets (third experiment)			
2	38	0	..
2	39	0	..
10	40	5	40
2	41	7	41
5	42	5	42
0	..	3	43
3	44	2	44
2	45	7	45
0	..	2	46
0	..	1	47
0	.	1	48
Total 26	*Average 41.3*	*Total 33*	*Average 33.0*
Hatched May 3, and reared on curly-top sugar beets (third experiment)			
1	35	1	35
1	36	0	..
1	37	0	..
6	38	1	38
1	39	2	39
5	40	4	40
4	41	3	41
5	42	2	42
1	43	1	43
6	44	5	44
3	45	5	45
2	46	3	46
2	48	2	48
2	49	1	49
1	52	1	52
Total 41	*Average 42.3*	*Total 31*	*Average 43.2*

bers of adults were reared, a high mortality of nymphs occurred on healthy celery and more nymphs were reared to the adult stage on diseased than on healthy celery.

The average total duration of the nymphal stages of *Texananus spatulatus* was less on celery infected with the aster-yellows virus than on healthy plants. The time required for the completion of the nymphal stages on curly-top sugar beets was less than on healthy ones, although this leafhopper is not a vector of the curly-top virus.

A statistical analysis of the data on the duration of the nymphal stages of each adult *Texananus lathropi* and *T. spatulatus* showed no significance.

There was no evidence to show that the total duration of the nymphal stages of the beet leafhopper, *Eutettix tenellus,* is shorter on curly-top sugar beets than on healthy plants.

ACKNOWLEDGMENTS

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PLATE

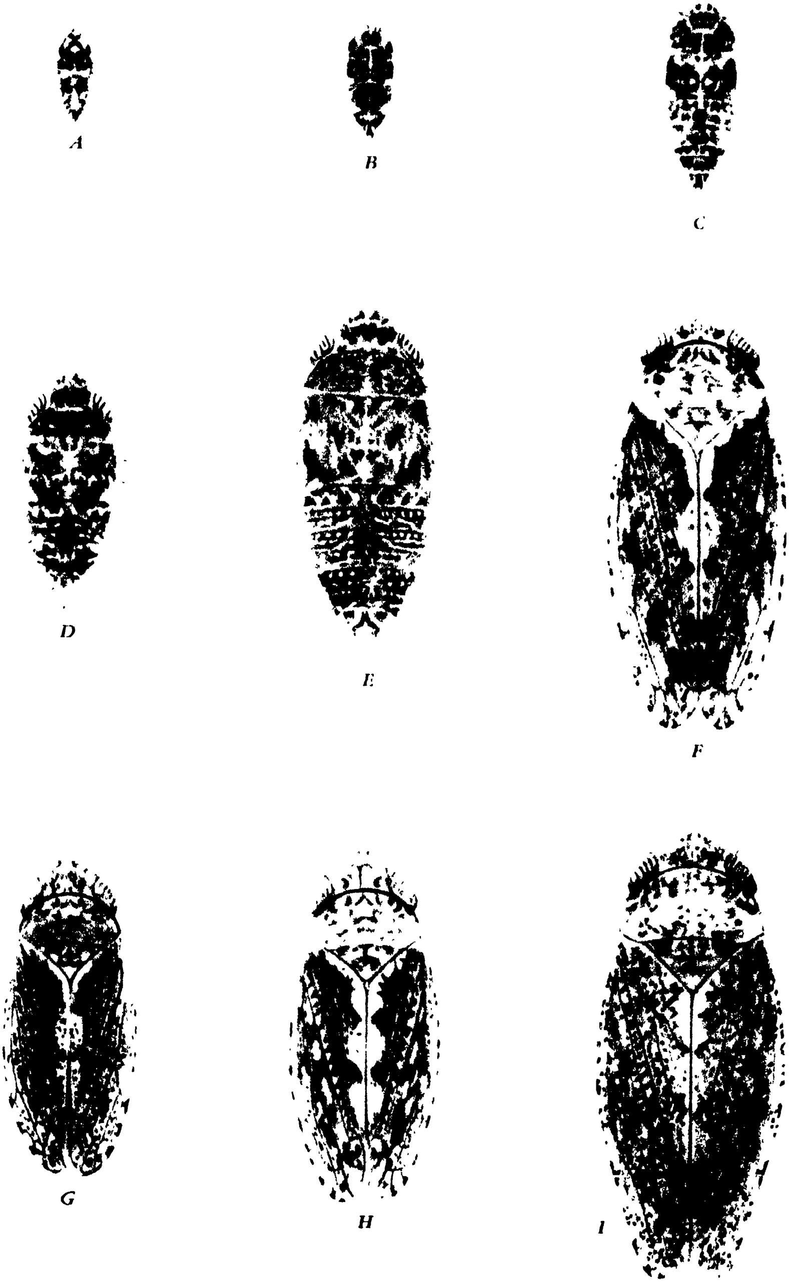

Plate 1.—Color patterns of nymphal instars and adults of *Texananus lathropi* Osborn and Lathrop: *A, B, C, D, E*, first to fifth nymphal instars respectively; *G*, male, and *H*, female. *F*, Male, and *I*, female of *T. denticulus* Osborn and Lathrop.

TRANSMISSION OF CALIFORNIA ASTER-YELLOWS VIRUS BY THE FIRST REPORTED LEAF-HOPPER VECTOR IN GYPONINAE

HENRY H. P. SEVERIN

TRANSMISSION OF CALIFORNIA ASTER-YELLOWS VIRUS BY THE FIRST REPORTED LEAF-HOPPER VECTOR IN GYPONINAE[1]

HENRY H. P. SEVERIN[2]

INTRODUCTION

Severin reported evidence that 10 leafhopper species and a biological race of one species (1940) transmit the California aster-yellows virus (1929, 1934, 1945).[3] The present paper deals with the transmission of the virus by *Gyponana hasta* DeLong. The characters, distribution, and food plants of this leafhopper have been discussed by DeLong and Severin (1946) in a companion paper.

An investigation was undertaken on the transmission of the virus to healthy celery and asters by single males and females and by varying numbers of adults. Experiments were conducted to determine the latent period and the retention of the virus in the adults, and whether this leafhopper could transmit the viruses of curly top and of Pierce's disease of grapevines. Life history studies were undertaken on the egg periods, egg-laying capacity, and duration of the nymphal stages. Measurements of various parts of the body were taken of each nymphal instar and of the adults.

METHODS

To determine the latent period of the virus in the adults, high populations of noninfective leafhoppers were reared on California common, or Chilean alfalfa, *Medicago sativa*, which is nonsusceptible to the disease. Infective leafhoppers were reared on celery infected with the virus. Life history studies were undertaken with nymphs which hatched from eggs deposited in the petioles of diseased celery. After determining the egg periods, the petioles in which oviposition occurred were cut into pieces a day or two before hatching and placed in stender dishes, the bottoms of which were covered with moist filter paper. Each nymph which hatched was transferred to an infected celery plant, and daily observations were made to determine each molt.

TRANSMISSION OF VIRUS TO CELERY

By Single Males and Females.—The efficiency of the vector in the transmission of the virus to healthy celery was determined with 50 males and 50 females. (The nymphal stages on diseased celery required an average of 84.5 and 81.0 days, respectively, during January and February, as shown in table 6.) Each leafhopper was kept on a healthy celery plant until symptoms of the disease developed, or during adult life if no symptoms appeared. Table 1 indicates that 18 per cent of the males and 12 per cent of the females caused infections.

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[1] Received for publication November 15, 1945.

[2] Entomologist in the Experiment Station.

[3] See "Literature Cited" for complete citations, referred to in the text by author and date of publication.

TABLE 1

Transmission of Virus to Successive Sets of Celery, by Varying Numbers of *Gyponana hasta*

Number of lots	Number of adults in each lot	First set of celery			Second set of celery			Third set of celery			Fourth set of celery			Total		
		Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected
50	1 male	50	9	18.0	..	..	....	..	..	....	..	..	....	50	9	18.0
50	1 female	50	6	12.0	..	..	....	..	..	....	..	..	....	50	6	12.0
25	5 males	25	6	24.0	22	3	13.6	22	2	9.1	..	..	....	69	11	15.9
25	5 females	25	6	24.0	24	2	8.3	23	0	0.0	2	1	50.0	74	9	12.2
25	10 males	25	15	60.0	22	0	0.0	20	0	0.0	7	0	0.0	74	15	20.3
16	10 females	16	5	31.2	15	1	6.7	15	0	0.0	1	0	0.0	47	6	12.8
25	20 males	25	12	48.0	23	6	26.1	22	1	4.5	22	0	0.0	92	19	20.7
17	20 females	17	2	11.8	17	3	17.6	16	1	6.2	16	0	0.0	66	6	9.1

By Varying Numbers of Adults.—An experiment was conducted with lots of 5, 10, and 20 adults which had completed the nymphal stages on diseased celery, to determine the percentages of transmission of the virus. Each lot of adults was kept on a healthy celery plant until symptoms of the disease appeared and then was transferred to a second healthy plant. If no symptoms developed within 30 days, the surviving adults were transferred monthly to successive healthy plants during adult life. The results obtained with the four sets of celery plants are shown in table 1. A comparison of the percentages of infections of the first set of celery plants shows that lots of 5 males and 5 females each infected 24.0 per cent of the celery; lots of 10 males and 10 females, 60.0 and 31.2 per cent, respectively; and lots of 20 males and 20 females, 48.0 and 11.8 per cent, respectively. The total percentages of infections produced by males were somewhat higher than those by females.

TRANSMISSION OF VIRUS TO ASTERS

By Single Males and Females.—The transmission of the virus to healthy asters was determined with 50 males and 50 females which had completed the nymphal stages on diseased celery. Each leafhopper was transferred and kept singly on a healthy aster plant during adult life. Table 2 shows that 1 of 50 females caused an infection and no infections were produced by the males. If this result is compared with the percentage of transmission of the virus to celery under the same conditions, it is evident that celery is more readily infected than asters (table 1).

By Varying Numbers of Adults.—A comparison was made of the infections produced by varing numbers of adults transferred to successive healthy asters every week or every 2 weeks. Each lot of leafhoppers was transferred to from 6 to 12 successive asters until the last adult died. A record was taken of the mortality of the adults. Table 2 records the results with 6 successive sets of asters. No infections were obtained with aster sets 7 to 12; hence, these are not included in the table. Lots of 10 males caused no infections of 30 asters inoculated every week, but 16.7 per cent of the asters were infected which had been inoculated every 2 weeks. In the weekly inoculations of successive asters with lots of 20 males, 6.7 per cent were infected, and 20.0 per cent of the asters were infected which had been inoculated every 2 weeks. Lots of 5 males infected 1.7 per cent of the asters inoculated every 2 weeks. Higher percentages of infections were obtained with lots of 10 and 20 males when asters were inoculated every 2 weeks rather than every week.

TRANSMISSION OF VIRUS TO TWO HOST PLANTS

Inoculations of one set of healthy celery and three successive sets of healthy asters for periods of 3 weeks were made with lots of 20 infective males which had completed the nymphal stages on diseased celery. Mortality records were taken with each transfer of the adults. The results (not tabulated) show that 11 of 21 of the first set of celery and the same number of the first set of asters were infected, or 52.4 per cent each; 6 of 21 asters of the second set were infected, or 28.6 per cent; and 1 of 19 asters of the third set was infected, or 5.3 per cent. The lower percentage of transmission of the virus to the third set of asters can be attributed to the mortality of the males. When these results

TABLE 2

TRANSMISSION OF VIRUS TO SUCCESSIVE SETS OF ASTERS, BY VARYING NUMBERS OF *Gyponana hasta*

Number of lots	Number of adults in each lot	First set of asters		Second set of asters		Third set of asters		Fourth set of asters		Fifth set of asters		Sixth set of asters		Total			Adults alive at end of 6 or 12 weeks
		Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Per cent infected	
50	1 male	50	0	..	..	..	..	..	..	..	..	..	..	50	0	0.0	....
50	1 female	50	1	..	..	..	..	..	..	..	..	..	..	50	1	2.0	....
	Weekly inoculations																
5	10 males	5	0	5	0	5	0	5	0	5	0	5	0	30	0	0.0	1 to 5
5	20 males	5	1	5	0	5	1	5	0	5	0	5	0	30	2	6.7	12 to 16
	Inoculations every 2 weeks																
10	5 males	10	0	10	1	10	0	10	0	10	0	10	0	60	1	1.7	1 to 4
5	10 males	5	1	5	1	5	1	5	0	5	1	5	1	30	5	16.7	2 to 7
5	20 males	5	1	5	1	5	1	5	1	5	1	5	1	30	6	20.0	4 to 10

are compared with those obtained in table 2, in exposures of 1 and 2 weeks to the first or second set of asters, and with the same number of leafhoppers, it is evident that larger numbers of infections were obtained when inoculations were made during periods of 3 weeks, again demonstrating that the periods of exposure are a factor in the transmission of the virus.

TABLE 3

LATENT PERIOD OF VIRUS IN FIVE LOTS OF FORTY FEMALE *Gyponana hasta*, WITH CELERY AS THE HOST PLANT

Days on infected celery	Successive plants inoculated	Plants infected	Per cent infected	Days after transfer on which successive infections occurred, including initial day on infected celery	Adults alive at end of 42 days
1	41	2	4.9	19, 40	20
1	41	1	2.4	20	23
1	41	4	9.8	24, 26, 32, 33	18
1	41	4	9.8	33, 35, 38, 39	16
1	41	1	2.4	35	16

TABLE 4

LATENT PERIOD OF VIRUS IN VARYING NUMBERS OF ADULT *Gyponana hasta*

Number of adults	Days on infected celery	Successive plants inoculated	Plants infected	Per cent infected	Days after transfer on which successive infections occurred, including initial 10 to 15 days on infected celery	Adults alive at end of 42 days
20	10	32	2	6.2	26, 38	10
20	10	32	3	9.4	28, 32, 35	6
20	10	32	2	6.2	34, 42	12
10	15	27	1	3.7	13	4
10	15	27	2	7.4	16, 29	2
10	15	27	5	18.5	25, 31, 35, 36, 40	2

LATENT PERIOD OF VIRUS IN ADULTS

The latent period of the virus was determined with 5 lots of 40 females. Each lot of leafhoppers was kept on a diseased celery plant for 1 day and then was transferred daily to successive healthy celery for a period of 41 days. The minimum latent period of the virus ranged from 19 to 35 days, as is shown in table 3.

Lots of 5 and 10 adults kept on infected celery plants for 1 day, and then transferred daily to healthy celery for 41 days caused no infections (not tabulated). It was decided to increase the period of exposure on diseased celery to 10 days, with 3 lots of 20 adults, and 15 days, with 3 lots of 10 adults. The number of infections obtained is indicated in table 4. One lot of 10 males produced 5 infections, compared with 4 infections by each of 2 lots of 40 females, as shown in table 3. The latent period of the virus cannot be determined accurately with exposures of 10 and 15 days on infected celery, since the virus may have been acquired on the first or succeeding days. Each of 2 lots of 20 and 10 adults failed to produce infections and were not tabulated.

RETENTION OF VIRUS BY SINGLE ADULTS

The retention of the virus was determined with single males and females which had completed the nymphal stages on diseased celery. Each leafhopper, after producing the first infection on a healthy celery plant, was transferred daily during adult life to successive healthy plants. The results are shown in table 5. The virus was retained for a period of 11 to 46 days by 2 males. Three males and 2 females produced only the initial infection. The period of the first infection is not included in the retention of the virus, since the adults were able to acquire the virus again.

TABLE 5

RETENTION OF VIRUS BY SINGLE ADULT *Gyponana hasta* WITH CELERY AS THE HOST PLANT

Days on first plant before symptoms developed	Plants inoculated after first infection	Plants infected after first infection	Per cent infected after first infection	Days after first infection on which successive infections occurred	Longevity of adults, days
			Five lots of males		
41	12	3	25.0	43, 44, 46	76
55	75	1	1.3	11	131
49	13	0	0.0	0	241
70	23	0	0.0	0	93
119	19	0	0.0	0	127
			Two lots of females		
28	28	0	0.0	0	121
36	8	0	0.0	0	150

ATTEMPTS TO TRANSMIT VIRUSES OF CURLY TOP AND PIERCE'S DISEASE OF GRAPEVINES

An attempt was made to transmit the curly-top virus by means of *Gyponana hasta*. One lot of 20 males was transferred daily, alternating with curly-top sugar beets and healthy beets, but no infections were produced. Five lots of 50 males or females were exposed to curly-top beets for 2 days and then were transferred daily to successive healthy beets during adult life. Thirty-two healthy beets were inoculated but no disease resulted. The last adult in each lot survived from 6 to 12 days, an average of 8.8 days on beets.

Eighteen tests were made with lots of 20 adults each to transmit the virus from Pierce's disease of grapevines to 5 healthy grapevines and to 5 healthy California common (Chilean) alfalfa and from alfalfa dwarf to 8 healthy alfalfa plants of the same variety. All inoculated grapevines and alfalfa remained healthy. The virus of Pierce's disease of grapevines is identical with the virus of alfalfa dwarf (Houston, Frazier, and Hewitt, 1945).

LIFE HISTORY

Egg Periods.—The egg periods of *Gyponana hasta* were determined with eggs deposited in the petioles of celery during January. Females at the egg-laying stage were confined in a cage enclosing a large celery plant for 1 day.

Eggs deposited during that day hatched over a period of 4 days; the maximum hatching occurred during the first day. The egg periods required from 22 to 26 days under greenhouse conditions.

Egg-laying Capacity.—To determine the number of eggs which a female deposits during her adult life, 1 female and 1 male were confined in a cage enclosing a large alfalfa plant. The eggs were allowed to hatch and the total number of nymphs removed from the cage would equal the egg-laying capacity, providing all of the eggs hatched. The nymphs were removed twice during each month from the cage. A total of 361 nymphs hatched.

TABLE 6

DURATION OF PERIODS BETWEEN MOLTS IN NYMPHAL STADIA OF *Gyponana hasta*

Date hatched	Duration of stadia, days					
	First instar	Second instar	Third instar	Fourth instar	Fifth instar	Total
			Males			
February 2	10	12	13	19	19	73
January 30	8	10	17	17	23	75
January 25	10	14	13	19	20	76
January 24	8	8	19	20	23	78
February 4	10	10	12	28	18	78
January 18	9	10	13	31	18	81
February 2	10	12	13	16	30	81
February 5	9	10	13	25	25	82
February 5	10	13	16	25	21	85
January 30	8	11	14	30	23	86
January 30	10	8	13	40	18	89
January 29	8	11	14	36	25	94
January 20	10	20	19	29	18	96
January 30	10	12	12	75		109
Average	*9.3*	*11.6*	*14.4*	*29.3*	*21.6*	*84.5*
			Females			
February 2	10	12	13	19	17	71
January 25	10	19	13	16	17	75
January 21	12	11	14	16	23	76
February 2	10	13	13	19	22	77
February 7	8	11	14	25	17	77
February 8	10	13	14	23	18	78
January 30	10	13	12	25	19	79
January 21	9	11	14	29	17	80
January 22	7	7	15	31	20	80
January 30	8	15	20	24	13	80
February 3	9	11	13	27	20	80
February 7	10	15	13	21	22	81
February 2	10	12	13	27	22	84
February 8	10	14	17	21	22	84
January 23	8	8	14	35	22	87
January 22	12	12	11	37	17	89
January 24	9	18	14	22	26	89
January 22	10	15	16	31	19	91
Average	*9.6*	*12.8*	*14.1*	*24.9*	*19.7*	*81.0*

Duration of Stadia.—The interval, or period, between molts (stages, or stadia) and the total duration of the nymphal stages were determined on diseased celery used as a food plant as indicated in table 6. The males required from 73 to 109 days, with an average of 84.5 days, to complete the nymphal stages; and the females from 71 to 91 days, with an average of 81 days. One nymph, a male, passed through four molts requiring 75 days to complete the fourth stadium, compared with 16 to 40 days, or an average of 25 days with males that passed through five molts. All females passed through five molts.

Measurements of Nymphal Instars and Adults.—Table 7 gives the average measurements of various parts of the body 1 day after hatching and 1 day after each molt of lots of 10 nymphs, 14 males, and 20 females. Using data from table 7, each instar can be determined from the range in measurements: the diameter of the head across the compound eyes, and the length of the head, thorax, and abdomen. Average measurements of the male and female leafhoppers that completed five molts show that the males are smaller than the females. The measurements of the male which molted four times (not tabulated) were as follows: diameter, head across compound eyes, 1.80 mm.; length, head and abdomen, 8.15 mm.; and length, head to end of wings, 8.15 mm. It is evident that the male, which molted four times, was not smaller than the average measurements of the males that passed through five molts.

TABLE 7

AVERAGE MEASUREMENTS IN MILLIMETERS OF INSTARS AND ADULTS OF *Gyponana hasta*

Nymphs and adults	Diameter of head across compound eyes			Length of head, thorax, and abdomen			Length of head to end of wings		
	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean
First instar....	0.63	0.61	0.62	1.54	1.35	1.44	...	....	....
Second instar...	0.84	0.76	0.79	2.67	2.24	2.39	....	...	....
Third instar....	1.07	1.00	1.03	3.51	3.19	3.31	..	...	....
Fourth instar...	1.43	1.31	1.38	5.05	4.59	4.80	....	....	....
Fifth instar....	1.88	1.67	1.80	6.83	6.02	6.59	...	....	....
Males........	1.85	1.75	1.80	8.71	6.89	8.12	8.58	6.89	8.16
Females......	2.15	1.89	2.01	9.58	8.10	8.94	9.55	8.50	9.15

Color of Nymphal Instars and Adults.—The nymph, upon hatching, is white, but later assumes a yellow tinge with reddish-brown areas on the posterior region of the head, on the pronotum, and on the anterior portion of the abdomen. The second and third nymphal instars are green and the fourth and fifth nymphal instars are pale green. These typical color patterns are shown in plate 1. The male and female are green.

Color variations of all nymphal instars occur, with the exception of the first instar as shown in plate 2.

SUMMARY

Fifty males and 50 females reared on diseased celery and tested singly on healthy celery caused 18 and 12 per cent infections respectively. One of 50 females reared on diseased celery infected 1 aster; no infection was produced by 50 males tested singly on asters.

The infections of successive celery plants in monthly transfers for 4 months by lots of 5 males and 5 females were 15.9 and 12.2 per cent respectively; by lots of 10 males and 10 females, 20.3 and 12.8 per cent respectively; and by lots of 20 males and 20 females, 20.7 and 9.1 per cent respectively.

A comparison of the transmission of the virus to successive asters by varying numbers of adults was as follows: by lots of 20 males in weekly inoculations, 6.7 per cent; inoculations every 2 weeks, 20.0 per cent; by lots of 10 males in weekly inoculations, 0.0 per cent; and for 2 weeks, 16.7 per cent; and by lots of 5 males during 2-week intervals, 1.7 per cent.

During periods of 2 weeks, inoculations of two host plants by lots of 20 males resulted in the following percentages of infections: first set of celery and first set of asters, each 52.4 per cent; second set of asters, 28.6 per cent; and third set of asters, 5.3 per cent. Asters are less readily infected than celery.

The minimum latent period of the virus in the adults ranged from 19 to 35 days.

The virus was retained for a period of 11 to 46 days. Most of the adults produced 1 infection and then apparently lost the infective dose.

Attempts to transmit the viruses of curly top and Pierce's disease of grapevines (alfalfa dwarf) by this leafhopper were failures.

Life history studies were made of the egg periods, egg-laying capacity, and duration of the nymphal stages.

The total duration of the nymphal stages of the males required from 73 to 109 days, with an average of 81 days; females required from 71 to 91 days, with an average of 81 days. One male passed through four molts, all others through five molts. Each instar can be determined accurately from tabulated measurements giving diameter of the head across the compound eyes, and the length of the head, thorax, and abdomen.

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PLATES

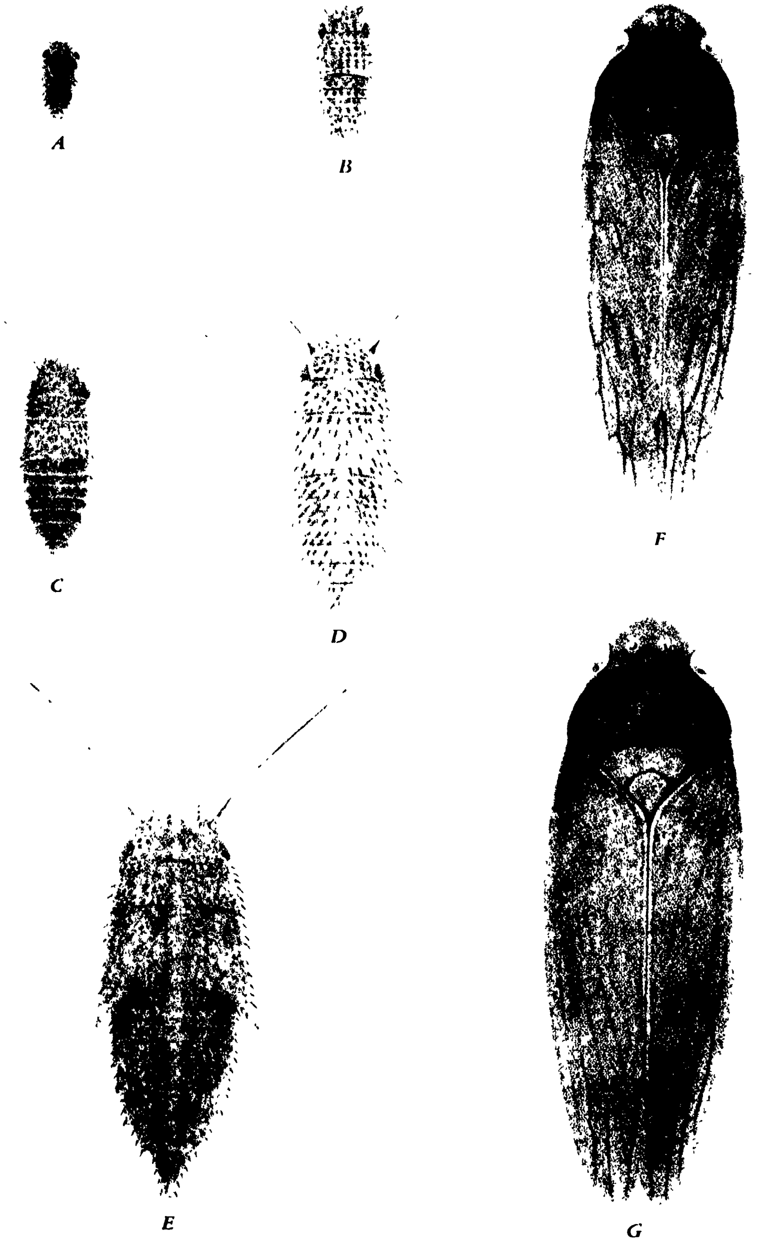

Plate 1.—Color patterns of nymphal instars and adults of *Gyponana hasta*: *A*, first nymphal instar, yellow in color with reddish-brown areas on the anterior portion of the abdomen, pronotum, and posterior region of the head; *B*, *C*, second and third nymphal instars, green in color; *D*, *E*, fourth and fifth nymphal instars, pale green; *F*, male and *G*, female, both green in color.

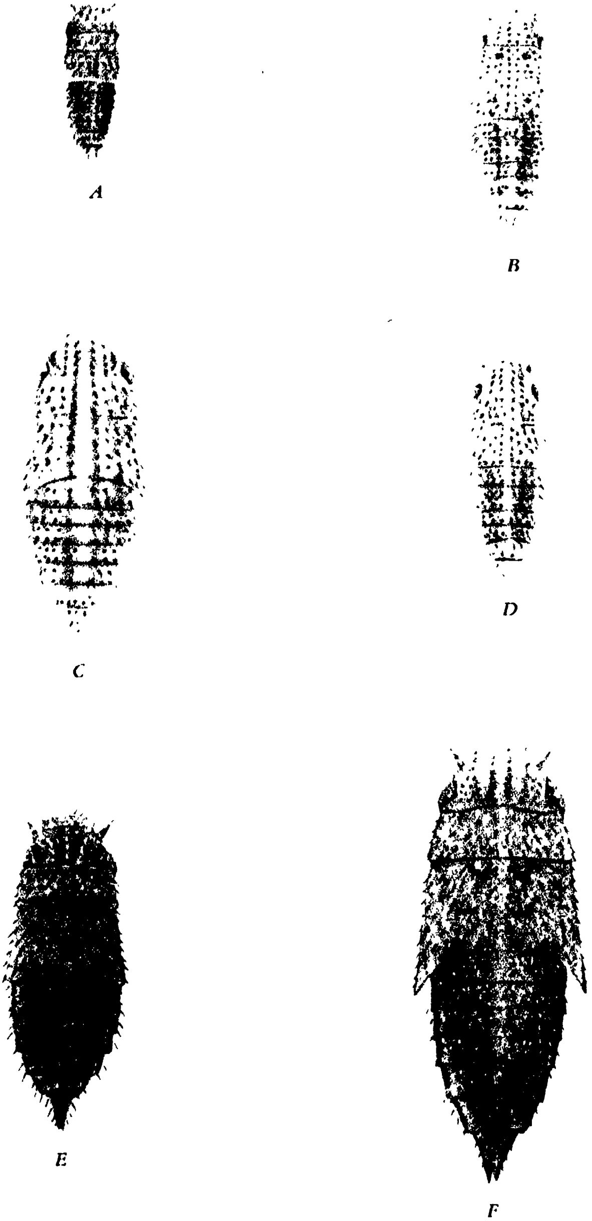

Plate 2.—Color variations of instars of *Gyponana hasta*: *A*, second instar; *B*, *D*, third instars; *C*, *E*, fourth instars; and *F*, fifth instar.

TAXONOMY, DISTRIBUTION, AND FOOD PLANTS OF *GYPONANA HASTA*, A LEAFHOPPER VECTOR OF CALIFORNIA ASTER-YELLOWS VIRUS

DWIGHT M. DELONG AND HENRY H. P. SEVERIN

TAXONOMY, DISTRIBUTION, AND FOOD PLANTS OF *GYPONANA HASTA*, A LEAFHOPPER VECTOR OF CALIFORNIA ASTER-YELLOWS VIRUS[1]

DWIGHT M. DeLONG[2] AND HENRY H. P. SEVERIN[3]

INTRODUCTION

SEVERIN has previously reported (1934, 1940)[4] that 3 species and a biological race of leafhoppers transmit the California aster-yellows virus. In two recent papers DeLong and Severin (1945) and Severin (1945) added 6 species of phlepsid leafhopper as vectors of this virus. The present paper deals with the characters, distribution, and food plants of one additional leafhopper vector, *Gyponana hasta* DeLong; in a companion paper, Severin (1946) discusses the transmission of the virus by this, the first reported leafhopper vector in the subfamily Gyponinae.

CHARACTERS, DISTRIBUTION, AND FOOD PLANTS

For several years *Gyponana hasta*, recently described (DeLong, 1942), has been confused with and identified as *Gyponana angulata* Spangberg, which it resembles very closely in general appearance and in morphologic structures.

Gyponana hasta is one of the few species of this genus with an angled vertex. This species is similar to *G. angulata* in general appearance, and can be distinguished from it only by the shape of the style and aedeagus. Its length is 7.5 to 9 mm.

The vertex is somewhat strongly produced and bluntly angled, and is two thirds as long at the middle as the basal width between the eyes.

In color it is somewhat variable, but usually is dull yellow with a tinge of green. The vertex, pronotum, and scutellum (plate 1, *A* and *B*) are often definitely yellow. There are no dark nor definite color markings.

The female last ventral segment has a posterior margin which is broadly, concavely excavated, with a slight median notch (plate 1, *C*).

The male plates are elongate, blunt at the apex, and with a slight bulge on the outer margin near the middle (plate 1, *D*). The aedeagus (plate 1, *E*) is long and somewhat slender, with a pair of short terminal, lateral processes which extend about one third of the distance to the basal curved part and are closely appressed to the body of the aedeagus. The style is sickle-shaped, with the apical half broadened at the base, then gradually tapered to a sharp-pointed apex (plate 1, *F*).

In comparison with *Gyponana angulata*, the styles are longer and the tapered part is more slender before the apex. The aedeagus is longer, narrower, with shorter lateral processes which are more closely appressed.

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[3] Entomologist in the Experiment Station.

[4] See "Literature Cited" for complete citations, referred to in the text by author and date of publication.

Geographic Range.—The species was originally described from specimens taken in Arizona in the following localities: Hualpai and Patagonia mountains, Sabino Canyon, Williams, Turkey Creek, and Granite Dells. In California, earlier records indicate that specimens were taken at Sacramento, Mint Canyon, Hamilton City, Mt. Shasta, and Freeport; in Texas, at Fort Davis and in Gillespie and Val Verde counties; in Oregon, at Mt. Hood; in Idaho, at Coeur d'Alene; in Washington, at Mt. Rainier; in Utah, at Snyderville; and in Missouri, at Cabool.

Distribution and Food Plants in California.—The localities and food plants of this leafhopper are as follows:

San Luis Obispo County: 1 pair was collected in a pasture at Santa Maria, June 12, 1942, by N. W. Frazier.

San Diego County: 1 nymph (a male after the last molt) was taken in a field of alfalfa, *Medicago sativa,* at Boulevard, June 7, 1942, by N. W. Frazier.

San Bernardino County: On June 5, 1942, at the entrance to Cajon Pass several adults were captured on squaw bush, *Rhus trilobata* by N. W. Frazier.

Imperial County: At Westmoreland, June 7, 1942, 1 pair was caught in an alfalfa field by N. W. Frazier.

Tulare County: On May 8, 1941, at Woodlake 8 adults were captured in an alfalfa field by N. W. Frazier.

Fresno County: Adults were obtained in sweepings of alfalfa fields in the vicinity of Sanger, Selma, and Biola by H. H. P. Severin, and at Palier on April 15, 1942, by N. W. Frazier.

Madera County: At Madera, October 9, 1941, a few adults were taken in an alfalfa field by H. H. P. Severin.

Merced County: On April 28, 1943, at Livingston, 1 male and 2 females were collected on sagewort, or mugwort, *Artemisia vulgaris,* by N. W. Frazier.

Stanislaus County: On July 2, 1942, 6 males and 4 females were obtained in sweepings of alfalfa fields at Newman by N. W. Frazier.

Napa County: At Dry Creek School, February 13, 1943, a pair was caught in general sweepings, but the host plant was not recorded by N. W. Frazier.

Sacramento County: In a locality known as "Sacramento Pocket," on September 12, 1941, 2 adults were taken in a field of Ladino clover, *Trifolium repens* L. var. *latum* McCarthy, by H. H. P. Severin.

Among economic plants, this leafhopper was collected on one occasion in a celery field near Terminus, in the Sacramento Valley.

The collection data indicate that this leafhopper overwinters in the adult stage.

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PLATE

GYPONANA HASTA

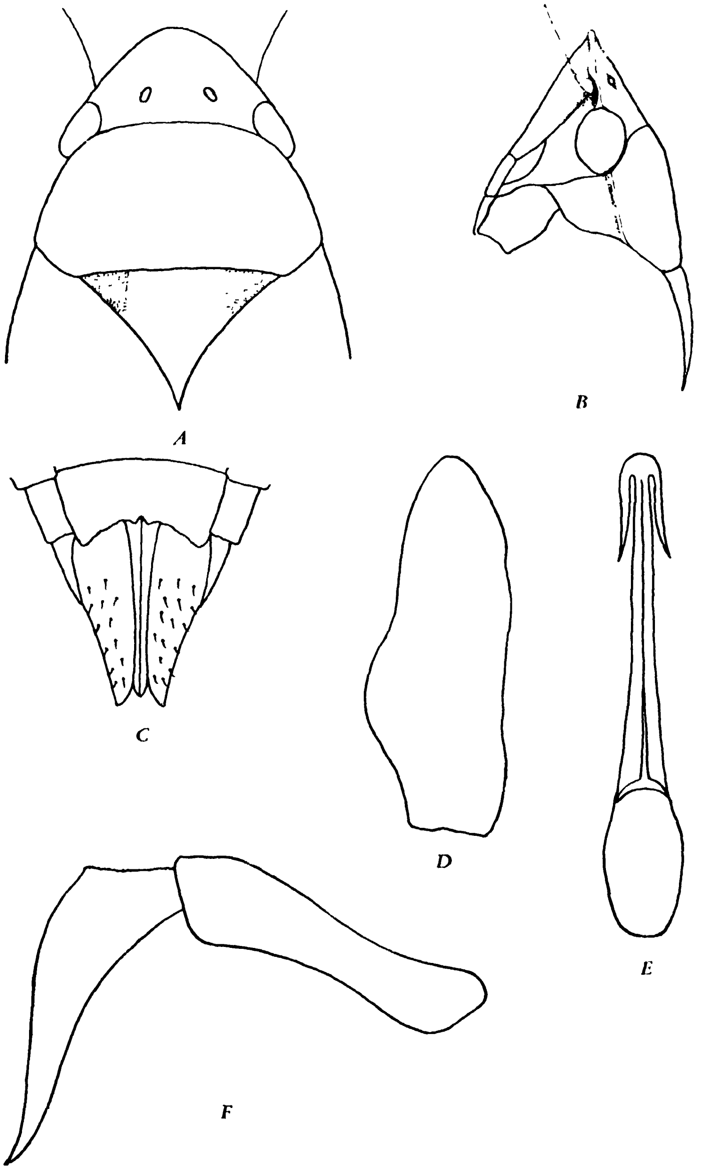

Plate 1.—*Gyponana hasta* DeLong: *A*, dorsal view of head, pronotum, and scutellum; *B*, lateral view of head, pronotum, and scutellum; *C*, ventral view of female external genital structures; *D*, ventral view of male plate; *E*, ventral view of male aedeagus; *F*, lateral view of male style.

H I L G A R D I A

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LYGUS BUG INJURY AND ITS EFFECT ON THE GROWTH OF ALFALFA[1]

LEE R. JEPPSON[2] AND G. F. MACLEOD[3]

HISTORY OF THE PROBLEM

THE GROUP of hemipterous insects belonging to the genus *Lygus,* of the family Miridae, has long been recognized as injurious to many agricultural crops and ornamental plants. In the past fifteen years two species of this genus—*L. hesperus* Knight and *L. elisus* Van Duzee—have been shown to cause flower drop and injury to pods and seeds of beans (Shull, 1933);[4] flower drop and shriveled seed in alfalfa (Sorenson, 1936, 1939; Shull, Rice, and Cline, 1934) and sugar beets (Hills, 1941; Hills, 1943); and shedding of the squares and other injury to cotton (McGregor, 1927; Ewing, 1929; Cassidy and Barber, 1939). As a result of these findings much information has been accumulated on the biology of these insects in relation to each of these host crops (Shull, 1933; Sorenson, 1939; and Smith, 1942).

The relation of lygus bugs to flower drop and shriveled seed of alfalfa has been the subject of extensive investigations by Sorenson (1932, 1939), Shull, Rice, and Cline (1934), Carlson (1940), and Stitt (1940), in which they have shown the importance of these insects in limiting alfalfa seed production.

Shull, Rice, and Cline (1934) were the first to suggest that lygus bugs might be a factor in alfalfa hay production. In their experiments, they put cages over four areas, each of which had from 17 to 20 plants. Two of these plots served as checks; on the others a population of bugs was maintained. An average of 17.5 per cent less hay was produced in the infested than in the check cages. Sorenson (1939) confined lygus bugs on alfalfa plants, and measured the lengths of the stems at various intervals. He found 8 to 35 per cent reduction in the length of the stems as a result of injuries caused by the bugs.

After the present study was under way, Carlson (1940) published investigations in which he measured the stem lengths of plants which had been grown in the field and kept free from lygus bugs by being dusted five to seven times a week with a pyrethrum-sulfur mixture. Upon comparing the stems of pro-

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[4] See "Literature Cited" for complete citations, referred to in the text by author and date of publication.

tected plants with those of naturally infested plants he found an average difference in length of 6.8 inches in 22 days of growth. He observed, however, an increase in the number of stems—from 24 on protected plants to 40 on infested plants—but the number of leaves on the stems were about equal. This would indicate that the insect injury resulted in more stems but shorter lengths. Carlson also found that the air-dried forage of plants continuously infested with lygus bugs weighed significantly more than that from plants uninfested from the prebud stage until seed harvest.

From this review of earlier work, it is apparent that the investigators are not agreed on the extent of injury done by this group of insects to the vegetative growth of alfalfa. Neither is it possible by field observation to determine whether or not there is definite reduction in yield of forage alfalfa attributable to lygus bugs. This paper reports the results of greenhouse studies on the effect of feeding by lygus bugs on the total growth of alfalfa plants, and the nature of the feeding injury to the plant tissues.

HISTOLOGICAL STUDIES OF LYGUS BUG INJURY TO ALFALFA PLANT TISSUES

It is well known that lygus bugs usually feed at the growing tips, the internodes, and on the flowers and seeds of alfalfa plants. Although Carlson (1940) has adequately studied the effects of lygus bug feeding on flower buds and seeds, no information was available on the effects of injury by these insects upon the vegetative growth of alfalfa plants. Accordingly, histological studies of injured and normal-growth shoots of alfalfa plants were inaugurated.

The alfalfa stems used to study the nature of the damage to the growing tips were taken from artificially infested plants. The stems of potted alfalfa plants were cut 2½ inches above the crown. The plants were immediately caged and given opportunity to grow until the stems reached a length of 2 to 10 inches—according to the height desired—at which time 20 to 25 lygus bugs were introduced into the cages. After the plants had been exposed for 12 hours the bugs were removed and the plants recaged. Two or three terminal buds were removed after 24, 48, and 96 hours, and fixed in formalin-acetic-acid-alcohol for histological study. The buds were imbedded in paraffin, sectioned by the ordinary methods, and stained with iron hematoxylin at pH 1.4 according to the technique described by Craig and Wilson (1937).

Carlson (1940) found a collarlike area of discoloration surrounding a small perforation at the point where the insect's stylets had penetrated. Complete disintegration had followed, and this had resulted in the death of the injured buds. Carlson observed that dead buds frequently remained attached to the floral axis, more or less retaining the size and shape they had at the time of injury.

In these studies disintegration and complete breakdown of the young ovary were indicated in sections made of injured alfalfa flower buds. Plate 1, *A*, shows a raceme with several young ovaries that were completely disintegrated. An uninjured raceme is shown in *B*. Sections made of apical growing regions, axillary stems, and leaf primordia show a similar disorganization of the tissue surrounding the puncture made by the insect's stylets, and a complete disintegration of a relatively large area of meristematic tissue at the growing regions—terminal and lateral buds (plates 2 and 3). Observations made of injured stems indicate that stem elongation continues, though somewhat retarded, unless the new growth is impaired by continued insect feeding. This increase in stem length appears to take place either by a substitution of lateral bud primordia for the normal growing tip or by the regeneration of a new growing region from uninjured meristematic cells near the area of injury. Pilkington (1929) found that a regeneration took place in *Vicia Faba* and *Lupinus albus* after a considerable portion of the terminal meristem was injured. She states, "It is probable that not only that part of the growing point which lies above the youngest primordia is capable of giving rise to a new apex, but any undamaged superficial area of the meristem either above or between the youngest leaf primordia."

In the limited number of cases of lygus bug injury observed by the writers, the terminal meristem appeared to be completely destroyed. The lateral bud primordia were stimulated into activity and later developed into the growing apex of the stem. Plate 3 shows a longitudinal section through an injured stem tip 4 days after the bugs were removed from the plant. The well-developed, uninjured axillary bud at the right has reached the same transverse plane as the terminal growing region which had been destroyed by the feeding of the insects. In normal alfalfa tips (plate 4) the axillary buds first appear as small groups of dark-stained meristematic cells at the axils of the leaf primordia. As these develop, the growth at the apical meristem increases the stem length to such extent that when the lateral buds reach the stage of development shown in plate 3 they are a considerable distance below the terminal growing region. The presence of a lateral bud on the same plane as an injured terminal indicates that it is in the process of being substituted for the normal growing tip. In plate 2, *B*, a new terminal apex has been formed at *a* after the terminal region at *b* had disintegrated as a result of feeding.

One of the characteristic symptoms accompanying an infestation of lygus bugs on alfalfa stems is the crinkled and misshapen leaves. Inasmuch as the bugs do not normally feed upon the leaves and as attempts to produce these symptoms artificially by confining bugs to the leaves have failed, it appears that these deformities are initiated in the leaf primordia. Disintegrated areas may be seen in plate 2, *A*, at *a*, surrounding the punctures made by the bug stylets in the young leaves which enclose the growing tip of the stem. Whenever these damaged areas occur in a young leaf, growth appears to be retarded in the area of the injury and the leaf develops unsymmetrically. This type of growth results in an irregular or deformed leaf. When the young leaves that surround the apical tips of alfalfa stems are examined several days after injury has occurred (plate 2, *A* at *b* and *B* at *c*), the beginning of such irregularities may be observed.

The histological studies of injury by lygus bugs to the growing points of alfalfa plants make it apparent that definite, measurable decreases in growth and in yield of forage should occur. To test this theory under field conditions would be difficult, if not impossible, because of the many complex factors involved. It was therefore considered desirable to conduct experimental studies under greenhouse conditions where both insects and plants could be subjected to some degree of control.

GREENHOUSE STUDIES ON THE EFFECTS OF LYGUS BUG INJURIES UPON THE GROWTH OF ALFALFA PLANTS

At the beginning of these studies it was recognized that success in measuring the effects of injury by an insect species on the growth increment of a plant depended upon the degree to which the environmental conditions and the genetic constitution of both the plant and the insects were controlled without greatly changing the growth characteristics of the plant. Special effort, therefore, was made to control growing conditions by selecting plants which showed similarity in native vigor of growth; by growing plants in water cultures with controlled nutrient solutions; and by confining the bugs within the type of cage that would influence to the least possible degree the growth rate of the alfalfa plants.

Experimental Methods.—The plants used in these experiments were started from selected common alfalfa seed. The seeds were sprouted and grown in sand culture until they reached an average height of about 3 inches. The young plants were then transferred to tanks containing water culture solution. The cultural methods were adapted from those used by Hoagland and Arnon (1938).

Shull (1933), Sorenson (1939), and others who have made studies on the life history of *Lygus hesperus* and *L. elisus* have reported difficulty in maintaining populations under artificial conditions. Attempts made by the present writers to build up a population in the laboratory failed to supply sufficient insects for the greenhouse studies; therefore the bugs used in these studies were collected from alfalfa fields with an insect net, and transferred by means of an aspirator.

It was recognized that the growth rate of a plant may be greatly influenced by the type of cage needed to confine small insects. For this reason special attempts were made to minimize and to measure the influence of the cage upon the plant growth rate.

Cages were constructed of a cellophane which was permeable to moisture. They were 1½ feet high and somewhat conical in shape. The smaller end was glued to the metal ring of a fruit jar lid and placed over the cork used to support the plant in the tank. Air was forced into each cage in order to increase ventilation and circulation so that excessively high temperatures might be avoided. The air was supplied from the laboratory air-pressure system, and led from the outlet to the cages through rubber tubing. In order to maintain uniform pressure, and to equalize the air to the various cages, a glass tube having a small terminal diameter was connected to the end of each rubber tube and inserted through a perforated cork fixed in the wall of the cage. A thermometer was placed in one of the cages in order to check the temperature at various times of the day.

At the close of the sixth growth period these cages were discarded because it appeared that the size of the cage near the base was of insufficient diameter to permit adequate air circulation at the crown of the plant. This defect had resulted in the condensation of moisture at the base of the cage, and the development of mold on some of the largest and apparently most healthy plants. This factor was thought to be responsible for the early death of some of the plants. New cages, 11 × 9 inches and 2 feet high, were constructed. These were of sufficient size at the base to cover four plants. The frames were made of wood and were covered, except at the base, with transparent, moisture-permeable cellophane. A strip of cotton was glued around the bottom of each cage to prevent the bugs from crawling under the edge. Air was forced into each cage, as it had been in the others, but the air flow was increased to allow for the increased volume of the new cages. These were used during the seventh and eighth growth periods of experiment A, and during the sixth and eighth periods of experiment B.

Even though a continuous air stream was introduced into these cages, the changes in environmental conditions, caused by enclosure, were sufficient to reduce materially the growth rate of the caged plants. A few plants in experiment A, which were not caged until 131 days after they had been placed in tanks, grew from six to nine times as rapidly as those which had been caged during the entire period. The weights of plant tops taken at each cutting after the plants were 131 days old showed that uncaged plants had produced two to three times as much growth as those caged. Weights of uncaged plant tops are not included in the tables of this paper. The controls refer to caged plants which were free from bugs.

The reduction in growth rate caused by the cages did not appear to be due to any one factor. The plants enclosed unquestionably were shaded somewhat by the cages themselves, as the cellophane cast a definite shadow; however, the majority of the uncaged plants were also partially shaded by the cages of surrounding plants. Another factor which may have contributed to the reduction in growth was the constricting effect of the base of the cage. An attempt to eliminate this factor by increasing the size of the cages failed to increase the growth increment of the plants. A third factor which may have retarded growth within the cages was the quantity of air reaching the plant. It is possible that insufficient air was supplied to the plant to permit normal exchange of gases at the leaf surfaces. Plant physiologists have shown that the amount of carbon dioxide may be the limiting factor in plant growth. Meyer and Anderson (1939) state that "Under natural conditions during the summer months in temperate regions the carbon dioxide concentration of the atmosphere is most frequently the limiting factor in photosynthesis for all photosynthetic tissues which are well exposed to light." No ready explanation is available of the limitation in growth of these alfalfa plants as a result of enclosure.

Experiment A.—In experiment A, 96 alfalfa plants were selected on the basis of uniformity of size, and placed in the tanks. These plants were permitted to grow for 60 days; at the end of that period they were rearranged so that the largest plants were placed in tank A and the smallest in tank H. Case histories were kept of each plant through nine growth periods. At the close of each period the plant stems were cut 2½ inches above the crown, weighed, and placed in sacks. They were then dried at 60° C for 24 hours, after which time they were stored at room temperatures until their weight became constant. The dry weight was then recorded.

In the first three growth periods the plants were left unexposed to insects so that they might become established. However, in each tank all plants, with the exception of two, were caged during the second and third periods. The fourth, sixth, seventh, and eighth periods were used to determine the influence of various numbers of bugs on each plant; during the fifth and ninth periods the plants were permitted to develop without injury in order to ascertain the influence of feeding, by the various bug concentrations, upon the following growth period. By the close of the ninth period some of the plants had died. It was also observed that differences in growth increment, due to the treatment the plant previously had received, were gaining magnitude; therefore, it was decided to discontinue the case histories.

Six treatments were made in each of the fourth, sixth, seventh, and eighth cuttings. During every growth period, each treatment was replicated twice in each of the 8 tanks. Because of the difficulty in determining the exact number of bugs alive in each cage, no attempt was made to maintain the original population throughout the experiment; no additional bugs were introduced after the original infestation.

TABLE 1

MEAN DAILY DRY-WEIGHT GROWTH INCREMENT OF PLANTS DURING THE FIRST 111 DAYS IN EXPERIMENT A*

Cutting	Age of plant, days	Mean daily growth increment, in milligrams, according to tank number								Average daily growth increments
		1	2	3	4	5	6	7	8	
First....	73	10.9	7.5	12.2	13.3	8.8	11.7	7.9	5.8	9.7
Second..	97	40.0	29.8	37.4	33.8	16.1	33.0	24.7	26.6	30.2
Third...... .. .	111	120.6	96.5	110.0	89.8	128.0	98.7	93.3	91.1	103.5
Average growth increment per tank *		57.2	44.6	53.2	45.6	50.9	47.8	41.9	41.2	. . .

* Each mean based on 12 plants in each tank. No significant differences.

Experiment B.—In experiment B, 108 plants were placed in 9 tanks and cultured as those of experiment A. During the first 42 days of growth, which comprised two growth periods, no cages or bugs were placed on the plants. During the third and fourth periods, single plants were caged and the treatments were replicated twice in each tank and randomized on a tank basis. Cages sufficiently large to cover four plants were used for the sixth and eighth periods, and only three treatments were made in each tank. The treatments were then randomized among the 9 tanks. Cages were removed from the plants for the fifth, seventh, and ninth periods in order to ascertain the influence of injury received by the plants as a result of bug feeding, on the subsequent growth of the plants.

Results.—The results were compiled on the basis of the daily growth increment of each plant. This was obtained by dividing the weight of each plant when harvested, at the end of the period of growth, by the number of days in the period. This will be referred to as growth rate, or increment, in the tables and the discussion.

A steady increase in growth increment was apparent in the 111 days that were allowed for the plants to become established in experiment A (table 1).

Analysis of the weight samples taken three times during this period shows that the growth rate of each plant within a single tank was proportional to the other plants in the same tank, and that the plants of one tank increased in growth increment in proportion to the plants of the other tanks.

The symptoms resulting from the injury caused by the lygus bugs vary according to the severity of the damage. The injured plants were darker in color, the leaves more ovate, crinkled, and misshapen than the leaves on healthy plants. In cases of extreme injury the leaves were very small, the internodes short, and the stems thin and crooked.

The growth rate during the fourth growth period was greatly reduced when lygus bugs were confined on a plant. Table 2 shows the mean growth increment of the 16 plants in the check and with each of four populations of bugs. Analysis of these data indicated that differences in treatments, when expressed either as green, or as dry weight, were highly significant. The least significant difference between means based on fresh weight was 161.89 milligrams, and on dry weight, 15.39.

TABLE 2

MEAN DAILY GROWTH INCREMENTS OF PLANTS INFESTED WITH LYGUS BUGS IN THE FOURTH GROWTH PERIOD OF EXPERIMENT A*

Number of bugs on each plant	Mean daily growth increment, in milligrams†		Per cent growth increments as compared with check plants		Per cent moisture content of plants
	Fresh weight	Dry weight	Fresh weight	Dry weight	
Control	696	100.4	100.0	100.0	85
2	260	45.1	37.4	44 9	82
4	298	44.7	44.4	44.5	85
8	198	33.7	28.7	33 5	83
16	116	16.8	16.6	16 7	85

* Each mean based on 16 plants.

† Least difference necessary for significance in weight at the 5 per cent level: fresh weight, 161.89; and dry weight, 15.39. At the 1 per cent level: fresh weight, 218.42; and dry weight, 20.77.

In general, the growth of the plants was more rapid when there were fewer bugs feeding on them. When 2 to 4 bugs fed on plants of this size (fourth period of experiment A) the growth rate was reduced to about 45 per cent of that of the check plants; and when 8 and 16 bugs were introduced, the growth rate was reduced to about one third and one sixth, respectively, as compared with untreated plants. There was no difference in the growth increment of plants exposed to the feeding of 2 as compared with that of 4 bugs (table 2). The mean differences in fresh weights of plants having 2 and 16 or 8 and 16 bugs on each plant were not of sufficient magnitude to be significant at the 5 per cent level, whereas the dry-weight differences were significant. The moisture content of the plants, however, varied no more than 3 per cent between any of the treatments, and plants subjected to the feeding of 16 bugs had as high percentage of moisture as the check plants. Differences in weights obtained between infested and uninfested plants are therefore not a direct result of the removal of the plant juices by these sucking insects, but are measurements of the top growth produced by the alfalfa plants. The data indicate that the primary cause of reduced growth is not a disturbance of water balance in the plant caused by removal of water through the feeding of the insects.

The results from the sixth growth period are shown in table 3. The decrease in growth increment, as a result of infestation, is not of the same magnitude as was shown during the fourth period. The variations in weights within treatments also were greater. As a result, an analysis of the fresh weights showed significant differences between bug populations at the 5 per cent level, whereas the results of dry weights were under this level for significance. There was, nevertheless, considerable reduction in growth increment when 8 and 16 bugs were confined with the plants. The amount of moisture in the plants upon which 8 or 16 bugs were confined was 4 to 5 per cent less than the checks. This difference is small and not statistically significant; therefore, it may be attributed to experimental error, although the point should not be entirely ignored, for it occurs also in the seventh period of experiment A (table 4) and the third and fourth periods of experiment B (tables 7 and 8, respectively).

TABLE 3

MEAN DAILY GROWTH INCREMENTS OF PLANTS INFESTED WITH LYGUS BUGS IN THE SIXTH GROWTH PERIOD OF EXPERIMENT A*

Number of bugs on each plant	Mean daily growth increment in milligrams†		Per cent growth increments as compared with control plants		Per cent moisture content of plants
	Fresh weight	Dry weight	Fresh weight	Dry weight	
Control	735	99.5	100.0	100.0	86
2	711	109.9	96.7	110.4	84
4	686	101.0	93.2	101.8	85
8	508	84.0	69.1	84.3	83
16	423	74.0	57.5	74.3	82

* Each mean based on 16 plants.

† Least difference necessary for significance in weight at the 5 per cent level: fresh weight, 221.45; dry weight, no significant differences.

TABLE 4

MEAN DAILY GROWTH INCREMENTS OF PLANTS INFESTED WITH LYGUS BUGS IN THE SEVENTH GROWTH PERIOD OF EXPERIMENT A*

Number of bugs on each plant	Mean daily growth increment in milligrams†		Per cent growth increments as compared with check plants		Per cent moisture content of plants
	Fresh weight	Dry weight	Fresh weight	Dry weight	
Control	430	70	100.0	100.0	83.7
2	380	64	88.3	91.4	83.2
4	338	56	78.6	80.0	83.4
8	270	52	62.6	74.3	80.7
16	310	57	72.1	81.4	81.6

* Each mean based on 16 plants.

† No significant differences.

The results showing growth increment of the seventh period (table 4) indicate a reduced rate of growth as the number of bugs was increased, except that when 16 bugs were introduced to each plant the growth rates of each plant averaged slightly more than with plants subjected to the feeding of 8 bugs. These differences between treatments, however, because of the degree of variation within each treatment are not of sufficient magnitude to be significant at the 5 per cent level. A small decrease in water content was indicated in the plants that produced the least growth.

TABLE 5

MEAN DAILY GROWTH INCREMENTS OF PLANTS INFESTED WITH LYGUS BUGS IN THE EIGHTH GROWTH PERIOD OF EXPERIMENT A*

Number of bugs on each plant	Mean daily growth increment in milligrams†		Per cent growth increments as compared with check plants		Per cent moisture content of plants
	Fresh weight	Dry weight	Fresh weight	Dry weight	
Control	381	76	100.0	100.0	80.0
2	373	71	97.9	93.4	80.9
4	427	75	112.0	98.7	82.4
8	383	64	100.4	84.2	83.2
16	310	62	81.4	81.5	80.0

* Each mean based on 16 plants.
† No significant differences.

TABLE 6

AGE OF PLANTS, INTERVALS BETWEEN CUTTINGS AND INFESTATION, AND LENGTH OF FEEDING PERIODS AS REFLECTED IN PERCENTAGE REDUCTION OF PLANT GROWTH IN EXPERIMENT A

Age of plants in days	Days between cutting and bug infestation	Number of days plants were subjected to injury	Mean percentage reduction in weight of plants when infested with varying numbers of bugs per plant							
			2 bugs		4 bugs		8 bugs		16 bugs	
			Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
135	3	20	62.6	55.1	55.6	55.5	71.3	66.5	83.4	83.3
177	13	8	3.3	10.4*	6.8	1.8*	30.9	15.7	42.5	25.7
203	12	14	11.7	8.6	21.4	20.0	37.4	25.7	27.9	18.6
238	8	27	2.1	6.6	12.0*	1.3	0.4*	15.8	18.6	18.5

* Increase.

The differences between treatments during the eighth growth period (table 5) were not sufficient to show significance when fewer than 8 bugs were introduced to the plants, but those exposed to 16 bugs showed a growth which was only 81 per cent of that of the check plants.

A comparison of the effect of lygus-bug feeding on plant growth at several intervals of development (table 6) indicates that more bugs were required to retard substantially the growth rate when the plants were young than when they were older and had acquired a larger root system. The first introduction of bugs was made only 3 days after the plants were cut; later introductions were made after 8 and 13 days had expired. The length of time in which the bugs were on the plants was less, in the last period, when their influence was of lower magnitude, than in the first period. This suggests that injury to the plant, immediately after cutting, more seriously reduced plant growth than after the plant stems had become well started in development.

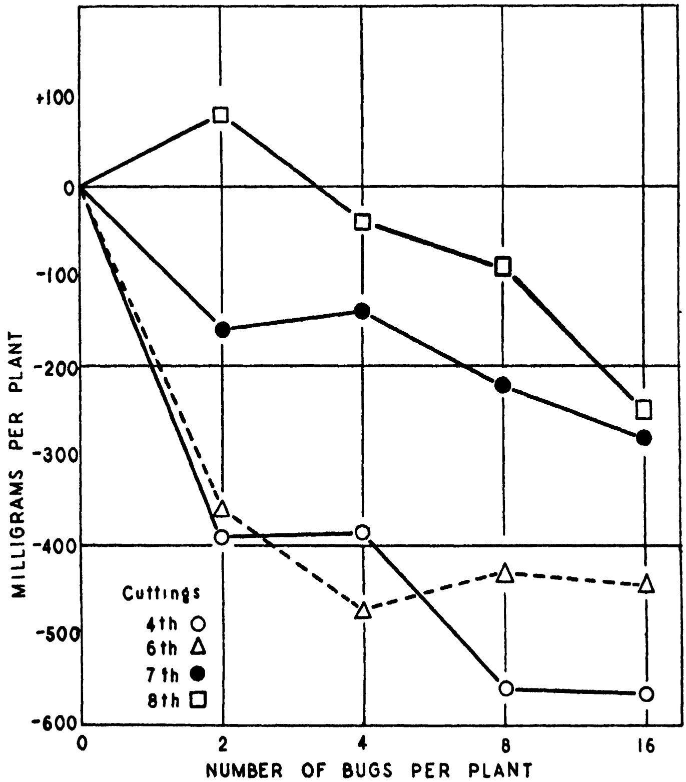

Fig. 1.—Reduction in daily fresh-weight growth increment in experiment A as a result of feeding by varying numbers of lygus bugs. The daily growth increments of each plant of the previous period were subtracted from those of the period shown, and the resulting means of each bug population were subtracted from those of the check plants. Each value represents the mean of 16 plants.

The decrease in growth rate caused by an increase in bug population, is more consistent when the results from each cutting are compared with those of the previous cutting (figure 1). These graphs also emphasize the lessened influence of corresponding populations of bugs as the plants increased in age.

The results from the second group of plants (experiment B) show a decrease in growth increment as the number of bugs is increased (much as those of the first group); however, the magnitude of the differences was not so great in the third and fourth periods as it was in the fourth period of experiment A. During the sixth to eighth periods the reduction of growth was greater when 2 or 4 bugs were introduced in the second group of plants than in the first group. During the third growth period of experiment B (table 7) there were no significant differences in plant growth as a result of feeding by different bug populations, when measured by either dry or fresh weight; however, mean weights of plants upon which 3 to 9 bugs were fed were less than the untreated plants.

TABLE 7

MEAN DAILY GROWTH INCREMENTS OF PLANTS INFESTED WITH LYGUS BUGS IN THE THIRD GROWTH PERIOD OF EXPERIMENT B*

Number of bugs on each plant	Mean daily growth increment in milligrams†		Per cent growth increments as compared with check plants		Per cent moisture content of plants
	Fresh weight	Dry weight	Fresh weight	Dry weight	
Control	521	71	100.0	100.0	86.3
1	532	72	102.1	101.4	86.4
3	392	62	75.2	87.3	84.2
6	431	67	82.7	94.3	84.4
9	344	67	66.0	94.3	80.5

* Each mean based on 18 plants.

† No significant differences.

TABLE 8

MEAN DAILY GROWTH INCREMENTS OF PLANTS INFESTED WITH LYGUS BUGS IN THE FOURTH GROWTH PERIOD OF EXPERIMENT B*

Number of bugs on each plant	Mean daily growth increment in milligrams†		Per cent growth increments as compared with check plants		Per cent moisture content of plants
	Fresh weight	Dry weight	Fresh weight	Dry weight	
Control	362	53	100.0	100.0	85.3
1	299	45	82.8	84.9	84.9
3	210	42	58 0	79.2	80.0
6	143	27	39.5	51.0	81.1
9	153	33	42.2	62.2	78.4

* Each mean based on 18 plants.

† Least difference necessary for significance in weight at the 5 per cent level: fresh weight, 148.09; and dry weight, 23.52. At the 1 per cent level: fresh weight 199.42; and dry weight, no significant differences.

Both the fresh and the dry weights taken from the fourth period (table 8) of experiment B indicate that when alfalfa plants were exposed to the feeding of 2 to 16 bugs the growth rate was significantly retarded. A difference of more than 148.09 milligrams fresh weight and 23.5 milligrams dry weight between any treatments is significant. Three or more bugs, therefore, reduced growth significantly over the check, and 6 and 9 over those plants upon which 1 bug was feeding (table 8). There was a variation of about 7 per cent in the moisture content between treatments, and plants fed upon by 9 bugs had the lowest percentage of water.

The results of the sixth and eighth growth periods also correspond to those of the other periods in reduction of growth increment when plants were exposed to the feeding activities of the insects. During the sixth period, treatment was made 4 days after cutting, and 4 bugs reduced growth to less than 70 per cent as much as that of the check plants (table 9). Analysis of the dry-weight increments indicated that the differences in means could not be entirely attributed to experimental error. At the eighth cutting the growth increment of plants exposed to 2 bugs was 63 and 74 per cent in fresh and dry weight, respectively—as much as in untreated plants.

It was not possible to count the number of living bugs present on a plant without removing the cage; therefore, the number which survived was not determined until the alfalfa was cut. No correlation could be made between growth and the length of time the insects survived on the plants; however, a relationship between the number of bugs surviving at the close of each period and the growth of the plants can be shown. Tables 10 and 11 show the dry-weight daily growth increment of all plants over which individual cages were placed, and the number of living bugs found at the end of the period.

TABLE 9

MEAN DAILY GROWTH INCREMENTS OF PLANTS INFESTED WITH LYGUS BUGS IN THE SIXTH AND EIGHTH GROWTH PERIODS OF EXPERIMENT B*

Number of bugs on each plant	Mean daily growth increment in milligrams		Per cent growth increments as compared with check plants		Per cent moisture content of plants
	Fresh weight	Dry weight	Fresh weight	Dry weight	
		Sixth period			
Control	240	44.7†	100.0	100.0	82.9
4	160	29.0†	66.7	68.3	82.5
		Eighth period‡			
Control	190	28.0	100.0	100.0	85.7
2	120	20.0	63.1	74.0	83.3

* Weights of controls represent the mean of 32 plants; all others, 64 plants.
† Difference between these means necessary for significance at 5 per cent level is 14.01.
‡ No statistical analysis was made for this period because a large number of plants was missing.

It appears from a study of these tables that the average weights of plants supporting the largest number of living lygus bugs, at the end of each treatment, was greater in 11 of the 20 treatments than the weights of plants on which the bug mortality was 100 per cent. At first glance it seems paradoxical that the results should indicate a reduction in growth rate when the initial bug population was increased and yet show less decrease in growth rate of plants which supported the largest percentage of the population throughout the entire growth period. Observations have shown that these lygus bugs feed largely on the meristematic tissue of the growing alfalfa stem. It is possible that 2 or more bugs continually feeding on the growing tips of an alfalfa plant may make the plant less suitable as food unless the plant is able to produce

TABLE 10

MEAN DAILY DRY-WEIGHT GROWTH INCREMENT OF PLANTS ARRANGED ACCORDING TO NUMBER OF LYGUS BUGS LIVING AT CLOSE OF PERIOD IN EXPERIMENT A

Number of bugs introduced per plant	Number of bugs living at end of period	Increments of growth in three periods, expressed in milligrams		
		Fourth period	Sixth period	Seventh period
2	0	56	48*	75
	1	43	86	60
	2	33	133	..
4	0	45*	125*	44
	1	42	97	59
	2-4	55*	86	..
8	0	40*	65*	..
	1 3	35	79	42
	4-8	40	89	37
16	0	40*	47*	35
	1-3	15	73	43
	4-8	12	46*	69
	9-16	24	101	81

* Means taken from only 1 to 3 plants.

TABLE 11

MEAN DAILY DRY-WEIGHT GROWTH INCREMENT OF PLANTS ARRANGED ACCORDING TO NUMBER OF LYGUS BUGS LIVING AT CLOSE OF PERIOD IN EXPERIMENT B

Number of bugs introduced per plant	Number of bugs living at end of period	Increments of growth in two periods, expressed in milligrams	
		Third period	Fourth period
1	0	43	46
	1	65	44
3	0	54	43
	1	57	48
	2-3	71	25*
6	0	49	29
	1-3	67	26
	4-6	80	40
9	0	75	37
	1-3	56	35
	4-9	81	32*

* Means taken from only 1 to 3 plants.

TABLE 12

MEAN DAILY GROWTH INCREMENTS OF UNINFESTED PLANTS IN THE FIFTH PERIOD OF EXPERIMENT A*

Number of bugs on plants in previous period	Mean daily growth increment of uninfested plants, expressed in milligrams†		Per cent moisture content of plants
	Fresh weight	Dry weight	
Control	1,130	131	88.4
2	1,410	182	87.1
4	1,360	181	86.7
8	1,820	249	86.3
16	1,430	192	86.5

* Each mean based on 16 plants.

† No significant differences. Least difference necessary for significance is 104 milligrams.

TABLE 13

MEAN DAILY GROWTH INCREMENTS OF UNINFESTED PLANTS IN THE NINTH PERIOD OF EXPERIMENT A*

Number of bugs on plants in previous period	Mean daily growth increment of uninfested plants, expressed in milligrams		Per cent moisture content of plants
	Fresh weight	Dry weight	
Control	1.343	202	84.9
2	1,548	235	84.7
4	1.214	183	85.7
8	1,188	172	85.6
16	1,080	166	84.6

* No statistical analysis was made because several plants were missing in each treatment.

TABLE 14

MEAN DAILY GROWTH INCREMENTS OF UNINFESTED PLANTS IN THE FIFTH PERIOD OF EXPERIMENT B*

Number of bugs on plants in previous period	Mean daily growth increment of uninfested plants, expressed in milligrams†		Per cent moisture content of plants
	Fresh weight	Dry weight	
Control	314	46.0	85.3
1	420	61.0	85.5
3	466	64.0	86.2
6	376	55.2	85.4
9	421	58.8	86.0

* Each mean based on 18 plants.

† No significant differences.

sufficient new meristematic tissue. Should this be possible, it would suggest one reason why trials made to maintain a population of bugs on a small number of plants in the laboratory have proved unsuccessful.

In order to ascertain the influence of lygus bugs feeding on growth of the succeeding period, the plants were allowed to grow without exposure to insects during the fifth and ninth periods of experiment A and the fifth, seventh, and ninth periods of experiment B (tables 12 to 15). An analysis of the growth increments of these periods indicated significant differences in only the fifth period of experiment A and the seventh period of experiment B. These differences did not indicate a carryover of bug injury from the previous period, although plants greatly retarded in growth when young, either by bugs or cages, did not equal the growth rate of unaffected plants for several cuttings.

TABLE 15

MEAN DAILY GROWTH INCREMENTS OF UNINFESTED PLANTS IN THE SEVENTH AND NINTH PERIODS OF EXPERIMENT B

Number of bugs on plants in previous period	Mean daily growth increment of uninfested plants, expressed in milligrams		Per cent moisture content of plants
	Fresh weight	Dry weight	
Seventh period			
Control	581	99.0	83.0
4	501	85.0	83.1
Ninth period			
Control	650	36 3	94 4
2	630	33.4	94.6

DISCUSSION

Injury to the tissues of alfalfa plants by lygus bugs appears to cause a local disintegration of cells surrounding the point of feeding. During the vegetative growing period of the stems these areas of injury were usually found to occur at the terminal growing point and lateral bud primordia. The meristematic tissue may be completely destroyed at these points. It is likely that stem growth continues in such cases by the stimulation of uninjured lateral buds to replace the injured growing points. A normal bud has several lateral bud primordia (plate 4), and a possible replacement of any of these for the injured terminal would permit an almost continual vegetative growth despite the large local areas of disintegration. When the plants are in the reproductive phase of growth there is no evidence of such a ready substitution. This suggests an explanation for the consistent injury shown by lygus bugs to seed production and for some of the variations in their influence on the growth rate of alfalfa. At cutting, the terminal bud and most of the lateral buds are removed; this leaves very little meristematic tissue upon which the bugs can feed, and a few of them could conceivably retard plant growth more than a large number after the stems have formed new terminal and lateral buds. The unusual degree of growth reduction in the two periods in which bugs were introduced three and four days after cutting (tables 4 and 7) may have been due to this factor.

The leaf deformity which is a characteristic symptom of infestation by lygus bugs appears to result from a penetration of the young leaves by the stylets. Carlson (1940, page 805) raises objection to this view because he found that "Damage to young flower buds, which are probably less sensitive to injury than leaf bud primordia, results in immediate and complete deterioration." The sections of buds in the vegetative stage of growth (plate 2) indicate that the lygus bug stylets penetrate through the young leaves which surround the growing regions of the stem. There appears to be only a slight deterioration of tissue surrounding the path of penetration of the stylets, whereas a large area is involved around the point of feeding, which seems to be principally on the flower buds and other growing regions (plates 1 and 2). The larger area of injury at the feeding point suggests that either a toxin was injected by the insects while feeding, which was only local in its effect, or the bugs by "probing around" and removing cell sap, injured large areas. This was followed by secondary pathological disintegration.

The methods and techniques used in the experimental studies permitted a considerable variation in the growth of the plants, which could be attributed to the influence of cages, nutrients, and undefinable causes; nevertheless, the results as presented in the various tables in this paper show that the injury caused by lygus bugs can be measured in terms of decrease in growth increment. In each of the groups of plants upon which 6 or more bugs were placed there was a substantial decrease in growth over that of the check plants. The populations introduced to the plant in these experiments should not be interpreted in terms of field populations since in five out of the eight growth periods the bugs were on the plants less than half of the period. Only one third of the bugs survived during the entire length of the growth periods, and it is likely that a high percentage of this mortality took place shortly after their introduction, as a result of injury in collecting and handling. On the other hand, the presence of a cage over the plant resulted in a marked decrease in growth, which suggests the possibility that the caged plants may not have been able to recover from injury as readily as the uncaged plants.

Carlson (1940, pages 809 to 811) found that plants continuously infested from the prebud stage to seed harvest time weighed significantly more than uninfested plants, and his photographs well illustrate the difference in growth. He states that "It is not known definitely, however, whether this excess growth results from a failure of the plants to set seed or from a stimulation due to *Lygus* injury." In the same paper, however, his photographs taken of infested and uninfested plants in the full-bloom stage, indicate that the uninfested plants had produced the greater growth. Although Carlson showed that lygus bug injury resulted in excessive branching and dropping of the flowers, he failed to point out the possibility that the greater weight of the infested plants may have resulted from a longer vegetative growing period. It is well known that the vegetative growth rate of alfalfa decreases as the flowers and seeds develop. It is more likely that the inhibition of reproductive growth by the lygus bugs resulted in a continuation of the vegetative growth over a longer period rather than in any stimulatory effects produced by them.

SUMMARY

Histological sections of alfalfa tips from stems 2 to 4 inches high, taken 2 to 4 days after being artificially infested with lygus bugs, showed large areas of cell disintegration of the terminal and lateral bud primordia.

Longitudinal sections of uninjured alfalfa tips 2 to 4 inches high showed lateral bud primordia in progressive stages of development at successively lower transverse planes than the terminal meristem, whereas infested alfalfa tips sectioned 4 days after having been infested with lygus bugs showed well-developed lateral buds at the same transverse plane as injured terminal buds. This condition indicates that a retardation of growth had resulted, with the lateral bud in the process of substitution for the injured terminal region.

Discoloration and disintegration of the tissue surrounding the point of penetration by the bug stylets through young protective leaves, which enclose the terminal meristem, were confined to a small area around the stylet punctures.

The daily growth increment of alfalfa plants, as measured by fresh or dry weight when compared with uninfested plants, was consistently reduced by 6, 8, or 16 bugs. Plants infested with 2 or 4 bugs were reduced 30 to 60 per cent in daily growth increment, as compared with uninfested plants, when the bugs were introduced within 4 days from the time of cutting.

In five of six periods, when 1 to 4 bugs were confined on alfalfa plant stems after they were 6 to 10 inches high, growth rate was not significantly less than with uninfested plants.

A decrease in growth as a result of an infestation of lygus bugs during one period did not substantially influence the growth rate of the plants during the succeeding period.

ACKNOWLEDGMENTS

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PLATES

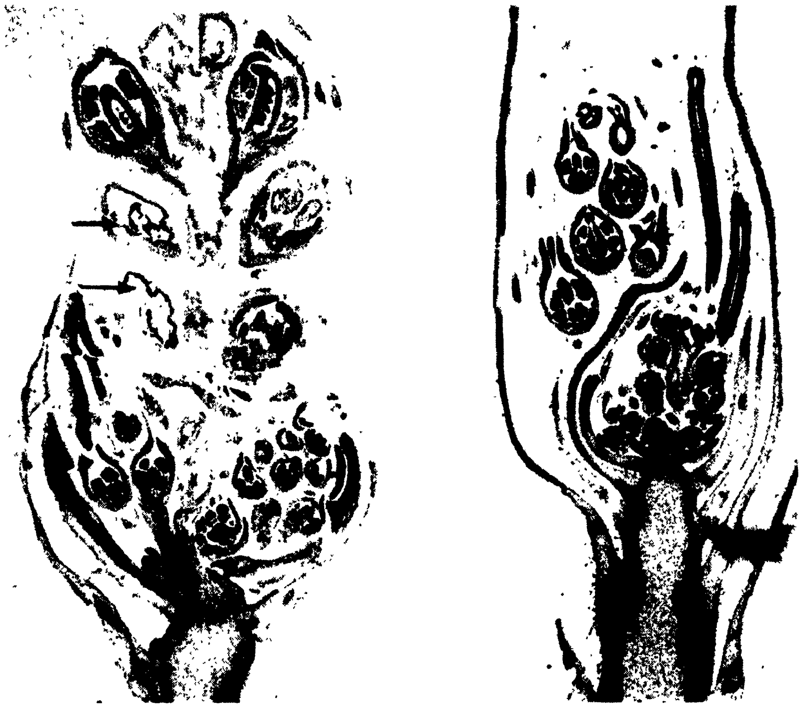

Plate 1.—*A*, Photomicrograph of a longitudinal section through a young alfalfa raceme injured by lygus bugs. Arrows show disintegrated ovaries. *B*, Longitudinal section through an uninjured young alfalfa raceme. (× 14.)

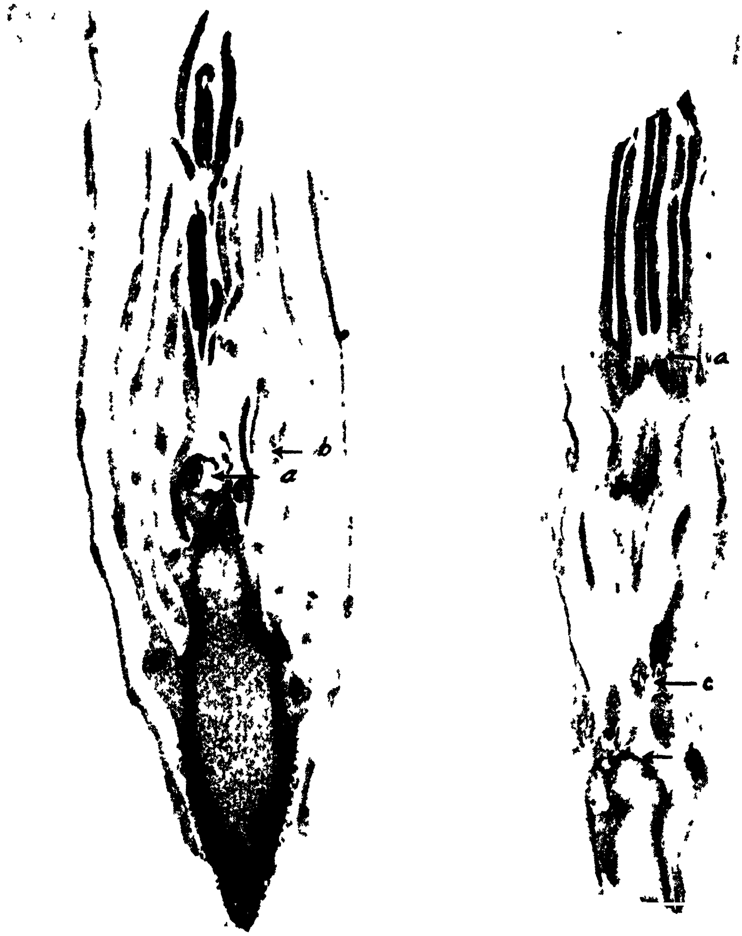

Plate 2.—A, Photomicrograph of a longitudinal section through an alfalfa bud in the vegetative growth stage showing (*a*) disintegrated terminal meristem resulting from lygus bug feeding and (*b*) path of stylets through protective leaf. *B*, Longitudinal section through a severely injured bud in vegetative growth stage 6 days after infestation, showing (*a*) formation of new uninjured terminal growing region after the old one (*b*) had been destroyed, and (*c*) injury to young leaf. (× 16.)

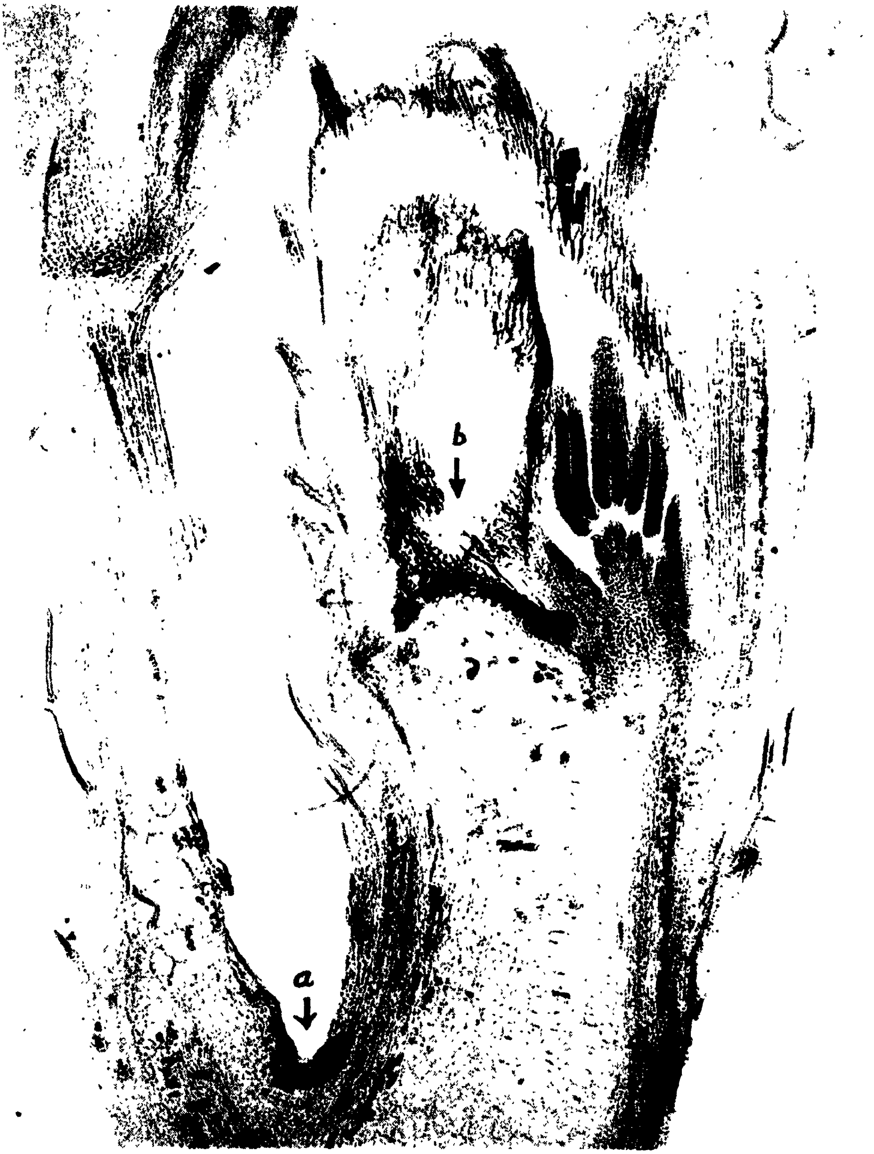

Plate 3.—Photomicrograph of a longitudinal section of an alfalfa bud injured by lygus bug feeding, showing disintegrated terminal growing region (*b*) and lateral bud (*a*). At the right is an uninjured lateral bud apparently in process of being substituted for injured terminal growing region. (× 43.)

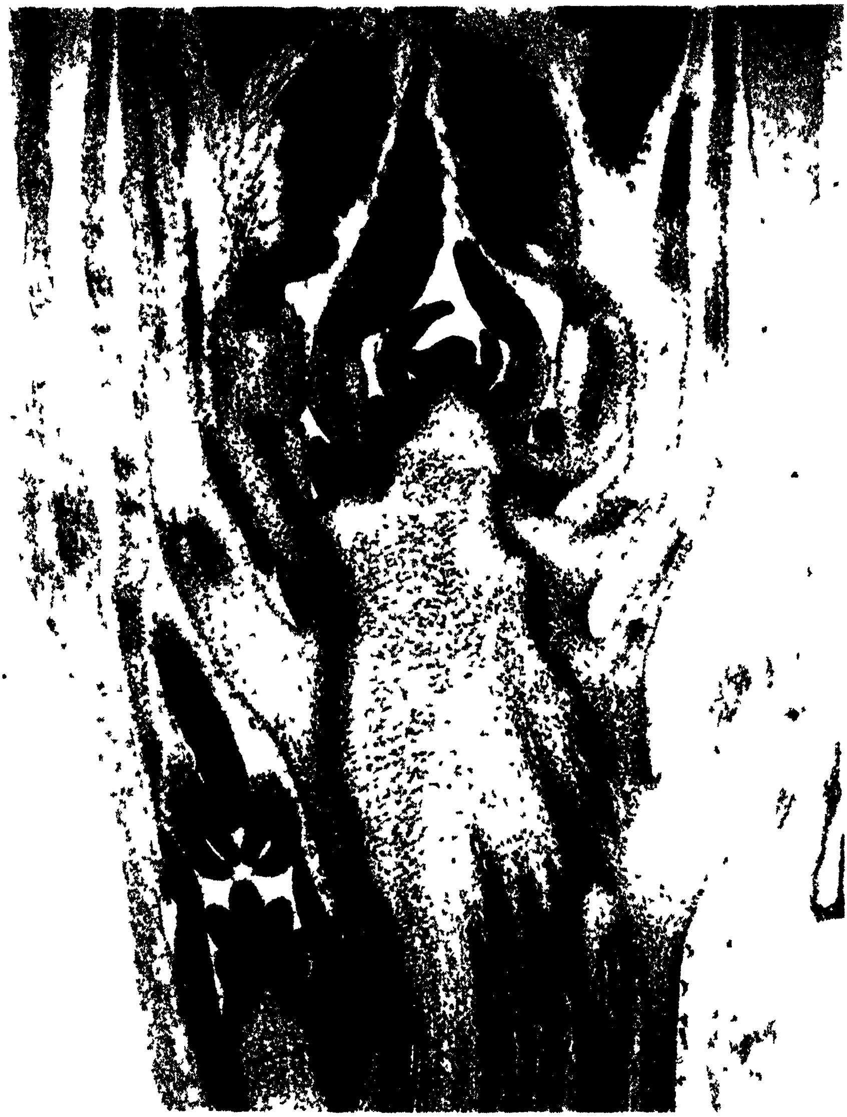

Plate 4 —Photomicrograph of a longitudinal section of a normal alfalfa tip Note growing apex with leaf primordia and successive stages in the formation of axillary buds appearing first as dark stained meristematic cells in the axils of young leaves (X 46)

THE WILLAMETTE MITE ON GRAPES

NORMAN W. FRAZIER AND LESLIE M. SMITH

THE WILLAMETTE MITE ON GRAPES[1]

NORMAN W. FRAZIER[2] AND LESLIE M. SMITH[3]

INTRODUCTION

FOR THE past three years the authors have observed extensive damage to grapevines by the Willamette mite, *Tetranychus willamettei* McGregor[4] throughout the grape-producing areas of Fresno and Tulare counties. Prior to these observations, many growers in these two districts were familiar with the injury, although not aware of its cause. During the past eight years the authors have seen occasional vineyards damaged by this mite also in Sacramento, Santa Clara, Stanislaus, San Joaquin, Yolo, and Sonoma counties. In the spring of 1938 and of 1939 the mite was unusually abundant in Stanislaus and San Joaquin counties, and in 1945 was abundant in Sonoma County.

The species was named and described by McGregor (1917), who collected the type material on leaves of white oak, *Quercus lobata,* at Oregon City, Oregon, August 22, 1915. He stated, "The presence of this species on oaks is revealed from a distance through the rusty appearance." It has also been reported on elm (Garman, 1940), and on pears and apples (Gentner, 1937; Newcomer, 1941; Overholser, Overley, and Allmendinger, 1944). Lamiman (1935) reported the presence of the Willamette mite on grapes, often in association with *Tetranychus pacificus* McGregor. He states that the Willamette mite "confines its attacks to spring and early summer, disappearing during hot weather."

SYMPTOMS OF INJURY

Affected vines in the summer and fall are a characteristic rusty yellowish green, especially the older, basal leaves. On weakened vines with short growth, all leaves may present such an appearance. The mites characteristically feed and produce colonies on the under surface of the leaf. This localized feeding produces injured areas which turn straw colored and then become scarious. This effect is visible in the upper leaf surface as chlorotic-green, straw, or straw-bronze discolorations. The initial feeding is confined to areas enclosed by the larger net veins, and a narrow strip of leaf tissue close to the larger veins remains green. The smaller net veins and often sections of the larger net veins, and the areas they enclose, lose their chlorophyll. During early stages of injury the chlorotic areas may be small and confined within fine net veins. They may consist of a very few spots scattered or grouped, or many spots evenly covering the entire leaf, in appearance sharply differentiated from the strip of normal green tissue banding the veins. This condition results in a faint chlorotic-green mottled appearance on the upper surface, somewhat similar to and easily confused with the mottle of Pierce's disease of grapevines, especially in late spring. Leaves that are nearly mature when first infested

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[1] Received for publication September 19, 1945.

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[4] The authors are indebted to E. A. McGregor for making the determination of the species.

show less intense mottling; the veins show no banding or only a slight suggestion of diffuse banding, which may be more clearly defined on shaded leaves.

When damage from Willamette mite occurs on newly opened buds and leaves, just as they unfold from the growing tip of the shoot, the injured areas—probably as a result of the rapid growth of these leaves after injury—are sharply defined. In other words, the junction of the injured, yellow tissue and the green, uninjured tissue forms a fairly sharp line. This delimiting of feeding areas can be used to distinguish the work of the Willamette mite from the damage caused by the Pacific mite, *Tetranychus pacificus.* This latter species does not produce injury in the early spring; instead, the damage it does appears on mature leaves in midsummer. As a colony increases in numbers and in size, the yellow spot on the leaf gradually expands so that the yellow center shades imperceptibly into the green, uninjured tissue.

The severity of injury is dependent upon mite population and growth factors of the vine. Injury may start with the first growth to appear in the spring and may keep pace with all subsequent growth during the remainder of the season. Or, it may affect only a few basal leaves of some canes, with injury apparently ceasing by midsummer; in the latter case, new succulent shoots that may grow in the fall are often attacked, especially those that are partially shaded. The effects on tender developing shoots may consist of dwarfing and deformation of leaves, angularity at nodes, shortened internodes, and spindling stems. The tips of the slow-growing axial shoots during summer or fall may be killed.

Vines showing the greatest degree of leaf injury are characteristically stunted and show a decided lack of vigor. There is a tendency for leaf incisions to be shallower and margins to be rolled downward. Canes are shorter, fruitfulness is impaired; clusters are fewer with less berries, and the berries are smaller, lack uniformity, are of poor quality, and appear to be somewhat delayed in maturity. Symptoms have been observed on the vinifera varieties Emperor, Ohanez, Muscat, Molinera, Tokay, Thompson Seedless, Carignane, Zinfandel, and White Malaga. They are basically similar, although some variations in expression occur on the several varieties.

The annual cycle has been completely studied only in the southern San Joaquin Valley. In the northern San Joaquin Valley, from about Modesto north, the hibernants could not be found.

SEASONAL CYCLE IN THE SOUTHERN SAN JOAQUIN VALLEY

The winter is passed in the adult stage by females. No males have been found to overwinter. The hibernating females are amber to lemon yellow—in contrast to the very much paler yellowish white or ivory color of the adults on the foliage; they can be found under the bark of three-year or older arms, mainly on the underside, singly or in small groups. Few have been found on the main trunk of the vines. They have been observed under outer layers of dead bark as well as in to the live bark. It is probable that feeding does not occur during the winter, although the mites are readily active when disturbed.

As the buds swell in the spring there is a tendency for the females to move slowly to the outer layers of bark, but not to the surface, along the arms and toward the spurs, remaining however on two-year or older wood and under loose bark. Shortly after the breaking of the buds and the beginning of expansion of the first leaves, the entire population moves out to the basal 2 or 3 leaves of the new shoots. In 1944, the first few Emperor buds opened on March 24; the migration of mites to the foliage took place between 3 and 7 days later. No observations could be made on the third to seventh day. On the eighth day, as no mites could be found remaining under the bark, it was assumed all had moved to the new shoots. In the same vineyard in 1945, the first buds broke on April 2; the migration of the mites took place during the eighth to fifteenth day thereafter. During the first 8 days the weather was cool, with 2 days of rain.

Buds do not break simultaneously on all vines in a vineyard, nor in all vineyards in a district. Some vines or vineyards may be delayed a number of days. On such late-breaking vines the mites were found to leave hibernation simultaneously with those on early-breaking vines, moving out to the swelling buds and collecting on the green portions of folded leaves exposed by the loosened bud scales.

In 1944, on April 6, about 9 days after migration of the mites to the leaves, the shoots ranged from 2 to 7 inches in length and had from 4 to 7 leaves, all immature. Thirty shoots examined under a binocular microscope showed the following total number of mites: 31 on the first basal leaf, 12 on the second leaf, 1 on a third leaf, and none on any of the more apical leaves. The adults spin a sparse web. The overwintering females become much paler after migration to the leaves. The eggs are tiny, round, transparent, and shiny and are laid anywhere on the under surface of the leaf, glued to the epidermis, leaf hair, or vein. The first nymphs were observed on April 19, about 7 days after the first eggs were found, but this does not necessarily indicate the exact egg period. On April 29, about 32 days after migration, examination of leaves under a binocular microscope showed a total of 23 overwintering adults, 151 eggs, 77 nymphs, and 4 first-generation adults. These first-generation adults were the first to be observed and indicated a nymphal period of about 10 days.

On approximately the fifteenth day after migration to the leaves, some of the overwintered adults leave the basal 2 or 3 leaves and make their way upward toward the younger leaves. A count made on May 8, about 53 days after migration from hibernation, revealed the presence of adults and eggs as far apically as the eleventh, eighth, and sixth leaf of three 14-leaf shoots. No overwintered adults were observed on these shoots. Totals of 1,605 eggs, 180 nymphs, and 279 adults were counted.

During both 1943 and 1944, throughout the summer and fall until at least November 1, frequent search showed eggs, nymphs, and adults to be present on the grape foliage, although no attempt was made to follow the complete life history in detail during those seasons. On November 30, 1944, a very few adults were still present on leaves, a number were observed migrating downward on the canes toward and on the arms and some were moving along trellis wires. Most of the mites on this date were already under the bark of the arms, since higher numbers could be found there than on the leaves and canes.

SEASONAL CYCLE IN THE NORTHERN SAN JOAQUIN VALLEY

In this area, the mites (all females) appear on the vines in the early spring just as the buds open. In heavy infestation, as many as 15 to 20 overwintered females may be found on a single bud. These mites lay their eggs as described above, but during May all stages usually disappear from the vines. The adults disappear first, then eggs, then nymphs, as indicated in the following averaged data for individuals per leaf on May 11, 1938: adults, 0; nymphs and larvae, 56; and eggs, 0.3. By May 20, no specimens remained. In another district, on May 14, microscopic examination of 11 injured leaves showed 9 leaves free from mites in any stage, 1 leaf with 2 adults and 76 young mites. On May 27, no mites in any stage could be found in this vineyard.

Repeated search for females hibernating under grape bark has failed to yield any specimens in the northern San Joaquin area. The locus of the mites during the winter is probably under the grape bark, as described above, but the sparse populations in the northern area make discovery difficult. In an attempt to find alternate host plants, mites were transferred to potted raspberry and bean plants, grown indoors, with potted grape plants as checks. The mites failed to survive on raspberry, but developed a large colony on bean and grape; they were discarded on July 6. Whereas the adults are pale yellowish white or ivory color on grape, they are yellow with large, granular, dark-green lateral pigmented spots which extend the entire length of the body when living on bean.

CONTROL

It is evident that this species causes considerable losses, especially during years of high populations, and warrants the development of specific control measures. Control studies of the Pacific mite have indicated a possible control of the Willamette mite. On February 10, 1939, a block each of the varieties of Carignane and Thompson were banded with a sticky material at the bases of the spurs. On April 4, examination of 15 suckers growing below the bands in the Thompson block gave 2 Willamette mites, and 6 similar suckers in the Carignane block gave 120 Willamette mites. No mites could be found above the sticky bands. The suckers were removed from the vineyard and destroyed on April 18, thus destroying the Willamette mite, and no damage occurred to the vines in either of these blocks that year. This banding and suckering method has been recommended for the control of the Pacific mite, but it may be even more effective against the Willamette mite. Since the latter emerges in great numbers in the early spring, when bands are effective, and since it is a much more active species than the Pacific mite, it runs blunderingly into the sticky material and is killed. The Pacific mite, on the other hand, avoids the bands.

SUMMARY

The Willamette mite is an important pest on grapes. As a result of injury to foliage caused by its feeding, vines appear to be weakened, with resultant loss in quantity and quality of crop. In the southern San Joaquin Valley, the females pass the winter in hibernation under bark on the arms of the vines, migrating to immature foliage in the spring shortly after the buds have broken.

In the northern San Joaquin, as well as in the Sacramento and Sonoma valleys, the mite has not been found in hibernation. Approximate observed periods of life-history stages of the first spring generation are as follows: 15 days from migration to foliage until the beginning of egg deposition; 7 days, egg period; and 10 days, nymphal period. Overwintered adults were still present on leaves 32 days, but not 53 days after spring migration to leaves. Eggs, nymphs, and adults were present on foliage as late as November 1, while on November 30, migration from foliage to hibernating quarters under bark was in progress. Banding the bases of the spurs with a sticky material may be a means of control.

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ACINOPTERUS ANGULATUS, A NEWLY DISCOVERED LEAFHOPPER VECTOR OF CALIFORNIA ASTER-YELLOWS VIRUS[1]

HENRY H. P. SEVERIN[2]

INTRODUCTION

IN FIVE PAPERS Severin (1929,[3] 1934, 1940, 1945, 1946) presented evidence that ten leafhopper species and a biological race of one of these species (1940) transmit the California aster-yellows virus. The present paper deals with the transmission of the virus by the leafhopper *Acinopterus angulatus* Lawson. The characters, distribution, and food plants of this leafhopper are discussed in a companion paper by DeLong and Severin (1946).

On May 27, 1935, adult *Acinopterus angulatus* were first collected on Spanish-clover, *Lotus americanus*, growing along the banks of the Salinas River near San Ardo. During the winter, all of these insects died. In 1936, other adults were taken in fields of alfalfa, *Medicago sativa*, near Soledad in the Salinas Valley; and since then high populations have been reared from them, and maintained on healthy and diseased celery. In a companion paper, DeLong and Severin (1946) give additional food plants of this leafhopper.

The color of the adults varies from brown (plate 1, *A*, *C*) to dark brown (plate 1, *B*) and yellowish brown (plate 1, *D*). A more detailed description of the color pattern of various parts of the body and wings is given in the accompanying paper by DeLong and Severin (1946).

An investigation was undertaken on the transmission of the California aster-yellows virus to celery or asters by single males and females, and by lots of varying numbers of adults. Experiments were conducted to determine the latent period and the retention of the virus in the adults. Attempts were made to transmit the viruses of curly top and Pierce's disease of grapevines by means of *Acinopterus angulatus*. One leafhopper described as a new species in the genus *Acinopterus* by Beamer (1944) failed to transmit the virus.

METHODS

Infective leafhoppers were reared during the nymphal stages on celery and, in one experiment, on aster infected with the California aster-yellows virus. In all the experiments reported in this paper, recently molted adults were

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[1] Received for publication August 16, 1945.

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[3] See "Literature Cited" for complete data on citations, referred to in the text by author and date of publication.

TABLE 1

TRANSMISSION OF VIRUS TO SUCCESSIVE SETS OF CELERY PLANTS BY VARYING NUMBERS OF ADULTS

Number of lots	Number of adults in each lot	First set of celery		Second set of celery		Third set of celery		Fourth set of celery		Fifth set of celery		Sixth set of celery		Total		
		Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Per cent infected
50	1 male	50	3											50	3	6.0
50	1 female	50	5											50	5	10.0
25	5 males	25	7	20	9	9	3	3	0	2	0	1	0	60	19	31.7
41	5 females	41	13	25	6	16	4	7	2	3	0	1	1	93	26	28.0
24	10 males	24	7	10	3	5	0	1	0					40	10	25.0
5	10 females	5	5	5	3	3	3	2	2	2	0	2	2	19	15	78.9
25	20 males	25	8	5	1	5	0	1	1	1	0	1	1	38	11	28.9
22	20 females	22	11	20	11	12	4	10	4	6	0	1	0	71	30	42.3

used. Males and females were removed daily from the cages after the last molt, so that the females had no opportunity to mate and to reproduce. The noninfective leafhoppers were reared on healthy celery and on alfalfa.

TRANSMISSION OF VIRUS TO CELERY

By Single Males and Females.—The efficiency of the vector in transmitting the virus was determined with 50 recently molted infective males and 50 females that had completed the nymphal stages on diseased celery; each was kept singly on a healthy celery plant until symptoms developed, or during adult life if no symptoms appeared. Three males and 5 females transmitted the virus to celery as shown in table 1.

By Varying Numbers of Adults.—In one experiment (table 1) lots of 5, 10, and 20 adults were transferred from diseased to healthy sets of celery. If symptoms developed, each lot of surviving insects was placed on a second healthy celery plant. If there were no symptoms, some lots were kept on the first healthy celery during adult life, whereas other lots were changed to successive healthy celery plants at irregular periods of exposure until the last adult died.

As the table shows, the total percentages of infections were as follows: lots of 5 males and 5 females, 31.7 and 28.0 per cent respectively; lots of 10 males and 10 females, 25.0 and 78.9 per cent respectively; lots of 20 males and 20 females, 28.9 and 42.3 per cent respectively. The percentages of infection did not increase progressively with lots of 5, 10, and 20 adults.

Successive Inoculations at Intervals of One and Three Weeks.—Tests were made on transmission of the virus by lots of 5, 10, and 20 males, each transferred weekly to 6 successive sets of healthy celery plants; and by lots of 10 females transferred at intervals of 3 weeks. As table 2 shows, the total percentages of infection in weekly inoculations by lot of 5, 10, and 20 males were 8.3, 18.3, and 15.0 per cent respectively, compared with 46.3 per cent with lots of 10 females at 3-week intervals. When the percentages of infections produced by 10 males in weekly inoculations are compared with those of 10 females transferred every 3 weeks, one sees that the period of exposure on healthy celery influences virus transmission.

TRANSMISSION OF VIRUS TO ASTERS

By Single Males and Females.—The efficiency of the vector in transmitting the virus was also determined with 50 males and 50 females, each kept singly on healthy asters until symptoms developed, or during adult life if no symptoms appeared. The virus was transmitted by 3 males and 6 females to 9 asters (table 3).

By Varying Numbers of Adults.—The transmission of the virus to asters was also determined with lots of 10 and 20 males. The adults were kept on healthy asters until symptoms developed; or, if there were no symptoms, each lot was transferred at irregular intervals to successive asters until the last adult died. As table 3 shows, the total percentage of infections was 14.3 per cent with lots of 10 and 20 males. Upon comparing these results with those shown in table 1 for lots of 10 and 20 adults, one notes that higher total percentages of infections occurred with celery than with asters.

TABLE 2

TRANSMISSION OF VIRUS TO SUCCESSIVE SETS OF CELERY PLANTS BY VARYING NUMBERS OF ADULTS AND VARYING PERIODS BETWEEN INOCULATIONS

Number of lots	Number of adults in each lot	First set of celery		Second set of celery		Third set of celery		Fourth set of celery		Fifth set of celery		Sixth set of celery		Total		
		Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Per cent infected
	Weekly inoculations															
10	5 males	10	2	10	0	10	2	10	0	10	1	10	0	60	5	8.3
10	10 males	10	2	10	2	10	1	10	2	10	4	10	0	60	11	18.3
10	20 males	10	2	10	1	10	1	10	3	10	2	10	0	60	9	15.0
	Inoculations every 3 weeks															
16	10 females	16	6	16	4	16	11	16	8	16	6	15	9	95	44	46.3

TABLE 3

TRANSMISSION OF VIRUS TO SUCCESSIVE SETS OF ASTERS BY VARYING NUMBERS OF ADULTS

Number of lots	Number of adults in each lot	First set of asters		Second set of asters		Third set of asters		Fourth set of asters		Fifth set of asters		Sixth set of asters		Total		
		Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Per cent infected
50	1 male	50	3	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	50	3	6.0
50	1 female	50	6	. .	. .	. .	. .	. .	. .	. .	. .	. .	. .	50	6	12.0
10	10 males	10	1	10	3	9	1	8	1	4	0	1	0	42	6	14.3
16	20 males	16	4	16	3	16	1	13	0	2	1	. .	. .	63	9	14.3

TABLE 4

INOCULATIONS OF SUCCESSIVE SETS OF ASTERS AT THREE-WEEK INTERVALS BY VARYING NUMBERS OF ADULTS

Number of lots	Number of males in each lot	First set of asters		Second set of asters		Third set of asters		Fourth set of asters		Fifth set of asters		Sixth set of asters		Total		
		Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Per cent infected
13	5	13	2	13	2	13	2	13	0	13	0	1	0	66	6	9.1
6	10	6	3	6	1	6	2	6	0	6	0	2	0	32	6	18.7

TABLE 5

TRANSMISSION OF VIRUS TO CELERY ALTERNATING WITH ASTERS IN WEEKLY INOCULATIONS BY LOTS OF FORTY MALES

Number of lots of 40 males each	First set of celery		First set of asters		Second set of celery		Second set of asters		Third set of celery		Third set of asters		Total asters		Total celery		Adults alive at end of 6 weeks
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	
1	1	1	1	0	1	0	1	0	1	1	1	0	3	1	3	2	28
1	1	1	1	0	1	0	1	0	1	1	1	0	3	2	3	1	21
1	1	0	1	0	1	1	1	1	1	0	1	0	3	1	3	2	28
1	1	0	1	0	1	0	1	1	1	1	1	0	3	0	3	2	37
1	1	0	1	0	1	0	1	1	1	0	1	0	3	0	3	1	24
Total	5	2	5	0	5	1	5	3	5	3	5	0	15	4	15	8	
Percentage	..	40.0	.	0.0		20.0		60.0		60.0		0.0		26.7	.	53.3	.

TABLE 6

Transmission of Virus to Asters Alternating with Celery in Weekly Inoculations by Lots of Forty Males

Number of lots of 40 males each	First set of asters		First set of celery		Second set of asters		Second set of celery		Third set of asters		Third set of celery		Total celery		Total asters		Adults alive at end of 6 weeks
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	
1	1	1	1	1	1	0	1	0	1	0	1	1	3	2	3	0	30
1	1	1	1	0	1	0	1	1	1	1	1	0	3	2	3	0	19
1	1	0	1	1	1	1	1	1	1	0	1	0	3	1	3	1	22
1	1	0	1	1	1	0	1	1	1	0	1	0	3	1	3	1	27
1	1	0	1	0	1	0	1	0	1	0	1	1	3	0	3	1	22
Total	5	2	5	3	5	1	5	3	5	1	5	2	15	6	15	3	..
Percentages	.	*40.0*		*60.0*		*20 0*		*60 0*		*20 0*		*40 0*		*40 0*		*20 0*	

Inoculations at Intervals of Three Weeks.—Successive asters were inoculated at 3-week intervals by lots of 5 and 10 males. According to table 4, the total percentages of infection by lots of 5 and 10 males were 9.1 and 18.7 per cent respectively. Upon comparing these results with those given for lots of 10 females in table 2, one finds higher total percentages of infection for celery than for asters.

TRANSMISSION OF VIRUS TO TWO HOST PLANTS

Transmission of the virus to celery alternating with asters, and asters alternating with celery, by lots of 40 males in weekly inoculations, were compared. For the first test the nymphs were reared to the adult stage on diseased celery; for the second test, on infected asters. As tables 5 and 6 show, 40.0 and 53.3 per cent of the total celery plants inoculated were infected; 20.0 and 26.7 per cent of the asters. Higher percentages of infection occurred with celery than with asters.

LATENT PERIOD OF VIRUS IN ADULTS

The latent period of the virus was determined with 5 lots of 80 previously noninfective males, which were kept on diseased celery for 1 day and then were transferred daily to healthy celery for 41 days. According to table 7, the minimum latent period of the virus ranged from 11 to 26 days; it averaged 18.4 days.

TABLE 7

LATENT PERIOD OF VIRUS IN FIVE LOTS OF EIGHTY MALES WITH CELERY AS THE HOST PLANT

Days on infected celery	Successive plants inoculated	Plants infected	Per cent infected	Days after transfer on which successive infections occurred, including initial day on infected celery	Adults alive at end of 42 days
1	41	4	9.8	11, 22, 29, 21	22
1	41	5	12.2	16, 24, 29, 35, 42...	42
1	41	7	17.1	17, 22, 32, 36, 37, 38, 41	36
1	41	6	14.6	22, 29, 35, 38, 40, 41	22
1	41	2	4.9	26, 31...	42

RETENTION OF VIRUS BY SINGLE ADULTS

Virus retention was determined with single adults that had transmitted the virus in tests of vector efficiency. After a leafhopper had produced the first infection, it was transferred daily to healthy celery or asters during adult life. One female kept on the first healthy celery for 67 days, after which symptoms developed, retained the virus for 51 days (table 8). The period of the first infection is not included in virus retention, since the adult was able to recover the virus again. Each of 2 females produced only the initial infection in celery, and likewise 2 males and 2 females caused only the initial infection in asters. The symptoms in 1 aster appeared only after 91 days, as determined by the greening of the flowers; in the other asters the cleared venation with yellow veinbanding developed in 12 to 30 days, or an average of 18.3 days.

Acinopterus parallelus, described as a new species by Beamer (1944), failed to transmit the California aster-yellows virus to healthy celery plants. The early nymphal stages were completed on *Artemisia vulgaris* and the later stages on infected celery. Four lots of 20 adults each were transferred from diseased to healthy celery plants during adult life, but not a single infection was obtained with 35 plants inoculated.

N. W. Frazier collected this leafhopper on June 11, 1942, near Fillmore, Ventura County, California.

TABLE 8

RETENTION OF VIRUS BY SINGLE ADULTS ON CELERY AND ON ASTERS

Lot no.	Days on first plant before symptoms developed	Plants inoculated after first infection	Plants infected after first infection	Per cent infected after first infection	Days after first infection on which successive infection occurred	Longevity of adults, days
With celery as the host plant						
1	67	95	3	3.2	41, 47, 51	162
2	85	85	0	0.0		160
3	90	16	0	0.0		106
With aster as the host plant						
4	12	35	0	0.0		47
5	13	8	0	0.0		21
6	30	40	0	0.0		70
7	91	26	0	0.0		56

ATTEMPTS TO TRANSMIT VIRUSES OF CURLY TOP AND PIERCE'S DISEASE OF GRAPEVINES

An attempt was made to transmit the curly-top virus to healthy sugar beets by means of adult *Acinopterus angulatus*. After 75 noninfective females reared on alfalfa had been kept on a curly-top beet for 2 days, they were transferred to 12 successive healthy sugar beets. In another test, 50 noninfective males were kept on a curly-top beet for 1 week and then transferred to 2 successive healthy beets. All inoculated beets remained healthy. The longevity of the males was 3 weeks on diseased and healthy beets.

No success was achieved in attempts to transmit the virus of Pierce's disease of grapevines to healthy grapevines and from alfalfa dwarf to healthy California common or Chilean alfalfa, *Medicago sativa*, by means of lots of 20 adult *Acinopterus angulatus*.

SUMMARY

Virus transmission by 100 males and females tested singly on healthy celery averaged 8 per cent. The average percentage of infection of successive celery at irregular intervals of inoculation by lots of 5 males and 5 females was 29.8 per cent; by lots of 10 males and 10 females, 51.9 per cent; by lots of 20 males and 20 females, 35.6 per cent. The total percentages of infection in weekly

inoculations of celery by lots of 5, 10, and 20 males were 8.3, 18.3, and 15.0 per cent respectively; by lots of 10 females during periods of 3 weeks, 46.3 per cent.

Infections produced by 100 males and females kept singly on healthy asters averaged 9 per cent. The total percentage of infections of successive asters during irregular intervals of inoculation by lots of 10 and 20 males was 14.3 per cent, whereas with lots of 5 and 10 males at 3-week intervals the percentages were 9.1 and 18.7 per cent respectively. Weekly inoculations of successive celery alternating with asters, by lots of 40 males, resulted as follows: celery 40.0 and asters 20.0 per cent respectively; asters alternating with celery, 26.7 and 53.3 per cent respectively.

The minimum latent period of the virus in adults ranged from 11 to 26 days, and averaged 18.4 days.

One female retained the virus for 51 days after producing the first infection; all others caused only the initial infection.

Acinopterus angulatus failed to transmit the virus of curly top and the virus which causes both Pierce's disease of grapevines and alfalfa dwarf.

Acinopterus parallelus, the only other species tested in this genus, failed to transmit the virus to healthy celery plants.

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PLATE

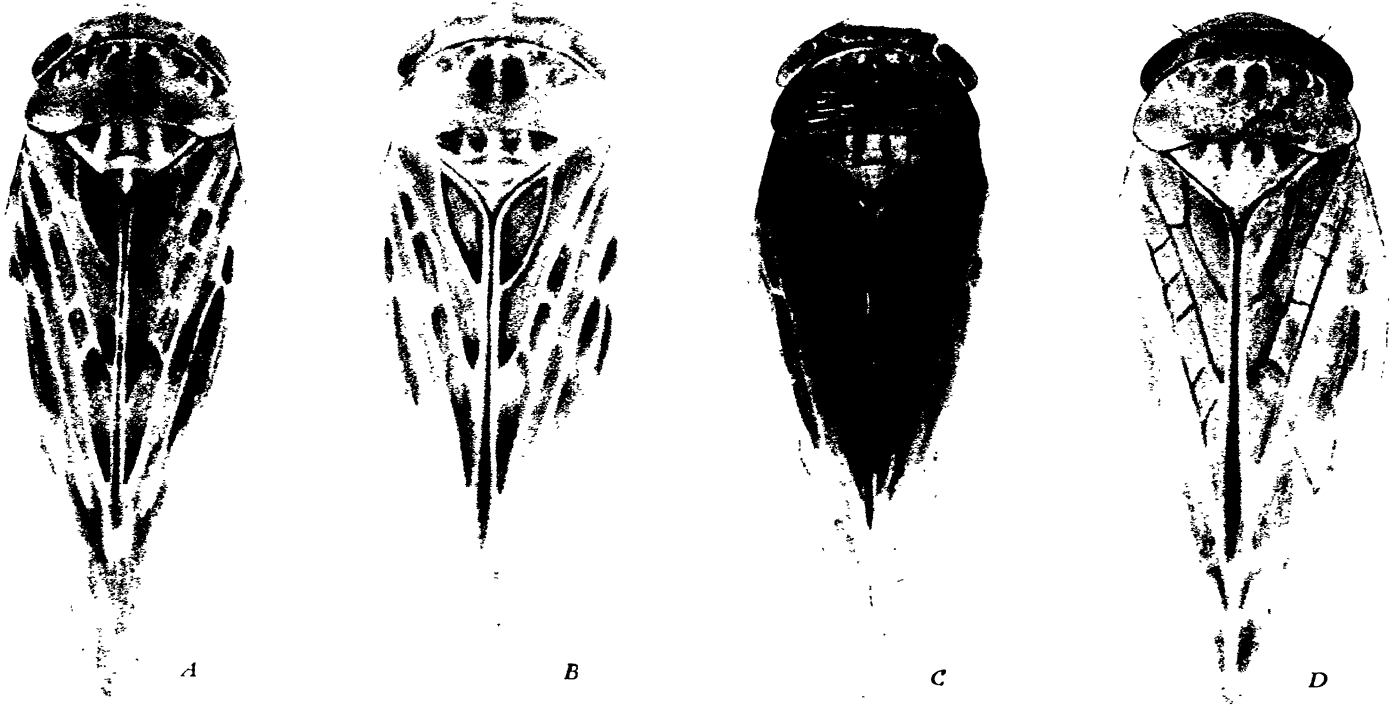

Plate 1.—*Acinopterus angulatus* Lawson: *A* male, and *B* female—light-brown forms; *C*, dark-brown male; and *D* yellowish-brown female.

TAXONOMY, DISTRIBUTION, AND FOOD PLANTS OF ACINOPTERUS ANGULATUS

DWIGHT M. DeLONG AND HENRY H. P. SEVERIN

TAXONOMY, DISTRIBUTION, AND FOOD PLANTS OF ACINOPTERUS ANGULATUS[1]

DWIGHT M. DeLONG[2] AND HENRY H. P. SEVERIN[3]

INTRODUCTION

SEVERIN HAS previously reported (1929, 1934)[4] that three species and a biological race (1940) of one of these species transmitted the California aster-yellows virus. In two papers, DeLong and Severin (1945, 1946) reported the characters, distribution, and food plants of seven leafhopper species. The present paper deals with the characters, distribution, and food plants of *Acinopterus angulatus* Lawson, a newly discovered vector of the virus. In a companion paper, Severin (1946) discusses the transmission of the virus by this leafhopper.

CHARACTERS, DISTRIBUTION, AND FOOD PLANTS

Acinopterus angulatus was described by Lawson (1922). He later redescribed it, in error, as *A. spatiosus* Lawson (1930) because of its variable external character.

The internal genital structures of the male will easily distinguish this species from other members of the genus. The distinguishing characters of this species are shown in plate 1.

This is a small blunt-headed species, light to dark greenish brown in color. The length is 5 to 7mm.

The vertex is short and broad, more than twice as wide at the base between the eyes as the median length, about one third longer at the middle than next to the eyes, the anterior margin broadly and bluntly angled, almost rounded. The elytra have an acutely pointed apex.

In color, the vertex, pronotum, and scutellum are greenish brown to yellowish brown. Three longitudinal lines on the scutellum, and the basal angles are lighter in color. The elytra are brown tinged with green, the veins are margined with darker brown. The face is brown, with portions of darker arcs.

The last ventral segment of the female is twice as long as the preceding. The posterior margin of the last ventral segment has distinct lateral angles between which the margin may be slightly concave or slightly produced with a small median notch.

The male plates are broad and more than twice as long as the basal width. They are only slightly narrowed toward the apices, which are broadly rounded and slightly divergent on the apical median margins. The style is bluntly pointed at the apex, then concavely rounded to form a lobe on the outer margin near the apex, narrowed by a deep concave excavation on the outer margin at the middle. The basal outer portion is long and tapered. The aedeagus is bifid at the base with a footlike process. A pair of basal processes are rather long,

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[4] See "Literature Cited" for complete data on citations, referred to in the text by author and date of publication.

curved laterally, and toothed at the apices. The terminal portion is long, tapered to a narrow, somewhat attenuated apex.

Geographic Range.—This species is common, and is widespread in distribution throughout California, Arizona, Texas, Mexico, Central America, West Indies, and South America.

Distribution and Food Plants in California.—The first population of this leafhopper was collected on Spanish-clover, *Lotus americanus,* growing along the banks of the Salinas River near San Ardo on May 27, 1935. During the winter all of the insects died. In 1936, other adults were next taken in alfalfa fields, *Medicago sativa,* near Soledad in the Salinas Valley. Nymphs and adults were abundant on wild licorice, *Glycyrrhiza lepidota,* in a locality known as the Sacramento Pocket. This leafhopper was captured in alfalfa fields in the Sacramento, San Joaquin, and Santa Clara valleys. Adults were occasionally taken in pastures of Ladino clover, *Trifolium repens* L. var. *latum* McCarthy, in the Sacramento Valley. The adults were also collected on pasture vegetation near the entrance of Pacheco Pass in the San Joaquin Valley and on weeds near Montara, San Mateo County. The collection data of this species of leafhopper indicate, up to the present time, that the preferred food plants belong to the Leguminosae, or pea family.

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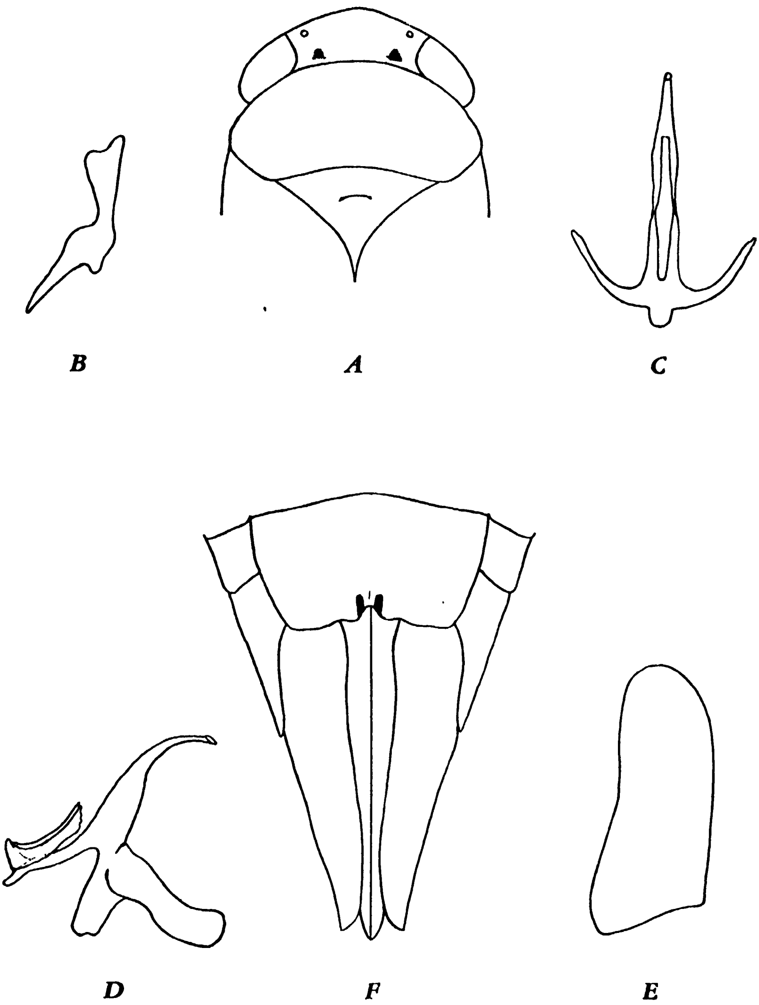

Plate 1.—Characters of *Acinopterus angulatus* Lawson: *A*, dorsal view of head, pronotum, and scutellum; *B*, ventral view of male style; *C*, ventral view and *D*, lateral view of male aedeagus: *E*, ventral view of male plate; *F*, ventral view of female external genital structures.

PLANT SYMPTOMS INDUCED BY FEEDING OF SOME LEAFHOPPER SPECIES

HENRY H. P. SEVERIN

PLANT SYMPTOMS INDUCED BY FEEDING OF SOME LEAFHOPPER SPECIES[1]

HENRY H. P. SEVERIN[2]

INTRODUCTION

IN A RECENT paper, Severin, Horn, and Frazier (1945)[3] described certain symptoms of curly top or aster yellows induced by the saliva of *Xerophloea vanduzeei* Lawson. On sugar beets it produced cleared veinlets, considered a reliable symptom of curly top. On asters it caused cleared venation with yellow veinbanding, stunting of the plants, development of axillary shoots from the bud in the axil of the leaves, and virescence of the flowers, all symptoms of aster yellows. The most striking effect produced by the feeding of the leafhoppers is breaking in color of the petals of asters.

In an investigation of a large number of leafhopper vectors of the California aster-yellows virus, 10 species induced symptoms on healthy China aster (*Callistephus chinensis*) and Golden Self-Blanching celery (*Apium graveolens* var. *dulce*) apparently by the saliva or by the feeding. A brief description of the symptoms produced by these leafhopper species follows.

TEXANANUS LATIPEX DELONG

In working on the life history of *Texananus latipex* it has been observed that some single noninfective nymphs induce cleared veins and veinlets with yellow veinbanding (plate 1, *A*) on the youngest leaf of healthy celery plants. In a later stage numerous small, green islands develop, surrounded by yellow areas (plate 1, *B*). Chlorotic areas appear on the intermediate leaves with scattered green islands. In the advanced stages, chlorosis gradually spreads on the inner and intermediate leaves until all of these leaves are yellow.

TEXANANUS LATHROPI OSBORN AND LATHROP

The symptoms produced by the feeding of some single noninfective nymphs of *Texananus lathropi* are similar to those described for *T. latipex*.

TEXANANUS PERGRADUS DELONG

With this species the symptoms on the youngest leaf of healthy celery caused by the feeding of the leafhoppers are cleared veins and veinlets with white veinbanding (plate 1, *C*), followed by mottling.

TEXANANUS SPATULATUS VAN DUZEE

The symptoms on the leaves of healthy celery induced by the feeding of nymphs and adults vary according to the populations of the leafhoppers. The first symptom is a clearing of the veins and veinlets (plate 2, *A*) on the youngest leaf of healthy celery, accompanied later with yellow veinbanding (plate 2, *B*). Yellow areas appear on the younger leaves (plate 2, *C*), and

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[3] See "Literature Cited" for complete data on citations, referred to in the text by author and date of publication.

chlorosis gradually spreads over the entire leaflets (plate 2, *D, E*), followed by a necrosis of the veins (plate 2, *F*). Large populations of nymphs and adults cause an outward rolling or curling of the leaflets, and chlorosis (plate 1, *D*).

The yellowing along the cleared veins followed by necrosis resembles the symptoms on celery leaves induced by the saliva of noninfective yellow willow aphids, *Carariella capreae* (Fabricius) as reported by Severin and Freitag (1938).

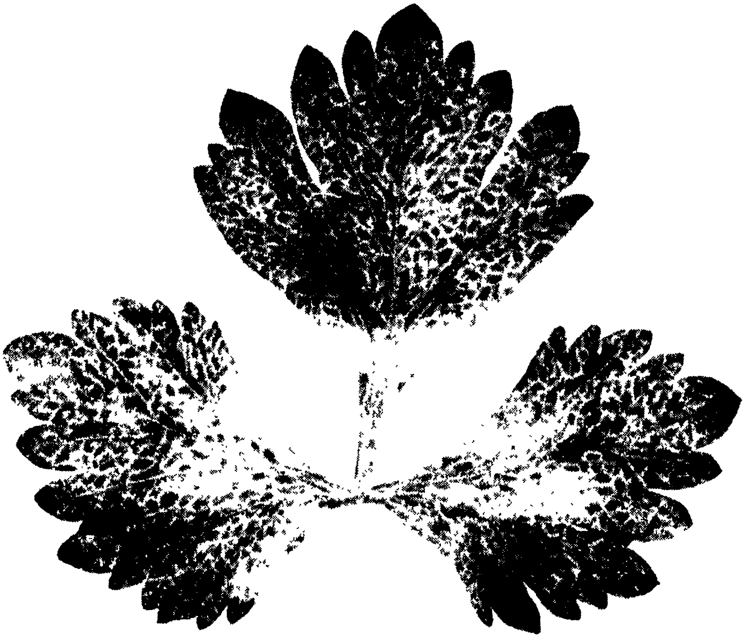

Fig. 1.—Cleared veins and veinlets on Golden Self-Blanching celery (*Apium graveolens dulce*) caused by the feeding of an occasional adult of *Acinopterus angulatus* Lawson.

The symptoms on the leaves of sugar beets nonsusceptible to aster yellows, are small yellow areas (plate 1, *E*) which gradually enlarge, followed by necrosis (plate 1, *F*).

GYPONANA HASTA DeLONG

The feeding of noninfective adults of *Gyponana hasta* on healthy celery causes a stunting and curving of the petioles of the younger leaves, but not a twisting and intertwining of the petioles as in celery naturally and experimentally infected with aster yellows (Severin, 1929). Noninfective short-winged aster leafhopper, *Macrosteles divisus* (Uhler), failed to recover the virus and transfer it to healthy celery or asters from celery showing symptoms produced by the feeding of noninfective *G. hasta*. When celery plants showing stunting and curving of the petioles induced by the feeding of this leafhopper were kept in insectproof cages, the petioles of the newly developing leaves were normal.

ACINOPTERUS ANGULATUS LAWSON

In testing the efficiency of virus transmission to healthy asters by single adults of this species an occasional plant showed cleared veins and veinlets with yellow veinbanding on a portion of an outer leaf (plate 3, *F*) resembling the earliest symptom of aster yellows. The youngest leaf, however, failed to show these symptoms. Noninfective short-winged aster leafhoppers failed to recover the virus from asters showing cleared venation on the older leaf and transfer it to healthy aster and celery plants. An occasional celery plant showed cleared veinlets (fig. 1) on the youngest leaf, caused by the feeding of a single adult.

COLLADONUS MONTANUS (VAN DUZEE)

The feeding of large populations of this leafhopper on celery plants causes a mottling of the leaflets (plate 3, *A*, *B*) and chlorosis which gradually spreads over the entire leaflets (plate 3, *C*, *D*, *E*). These symptoms may be induced by the drain of sap, or by the salivary secretion, or both.

COLLADONUS GEMINATUS (VAN DUZEE)

The symptoms on celery produced by the feeding of large numbers of this leafhopper are similar to those described for *Colladonus montanus*.

CLOANTHANUS IRRORATUS (VAN DUZEE)

The feeding of large populations of this leafhopper on celery plants causes cleared veins and veinlets with yellow veinbanding. Yellow areas appear on the intermediate leaves, and chlorosis spreads over the entire leaves.

IDIODONUS HEIDEMANNI (BALL)

Nymphs of this leafhopper species are yellow in color and each nymph in feeding on an aster leaf causes a yellow discoloration (plate 4, *A*), presumably serving as a protective resemblance. Each yellow area increases in size, followed by necrosis (plate 4, *B*). Since but 1 nymph will induce these symptoms, a toxic salivary secretion is probably involved.

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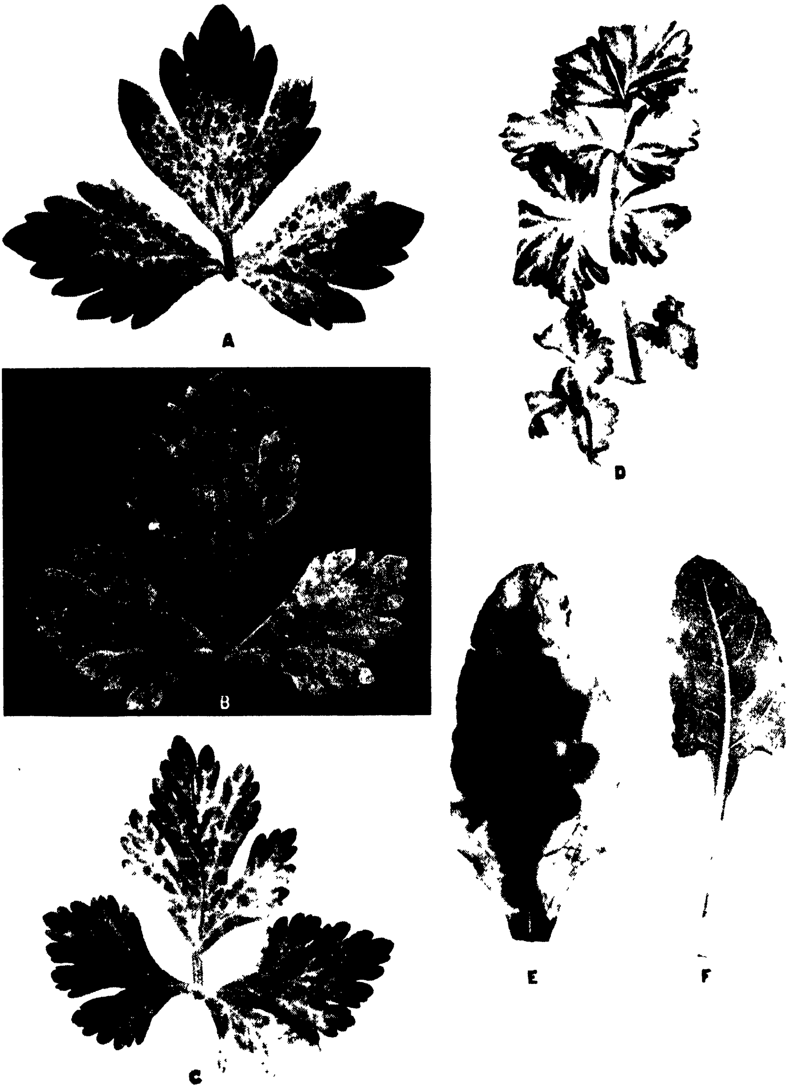

Plate 1.—Symptoms induced by the feeding of phlepsid leafhoppers: *A*, celery (*Apium graveolens dulce*) leaflets showing cleared veins and veinlets with yellow veinbanding, induced by the feeding of *Texananus lathropi* or by *T. latipex*; *B*, numerous small, green islands surrounded by yellow areas caused by *T. lathropi* or *T. latipex*; *C*, cleared venation, white veinbanding, and mottling produced by *T. pergradus*. Symptoms induced by *T. spatulatus*: *D*, outward rolling, curling, and chlorosis of celery leaflets; *E*, large yellow areas and necrosis of sugar-beet leaf; *F*, small yellow areas on blade and egg punctures in petiole of sugar-beet leaf.

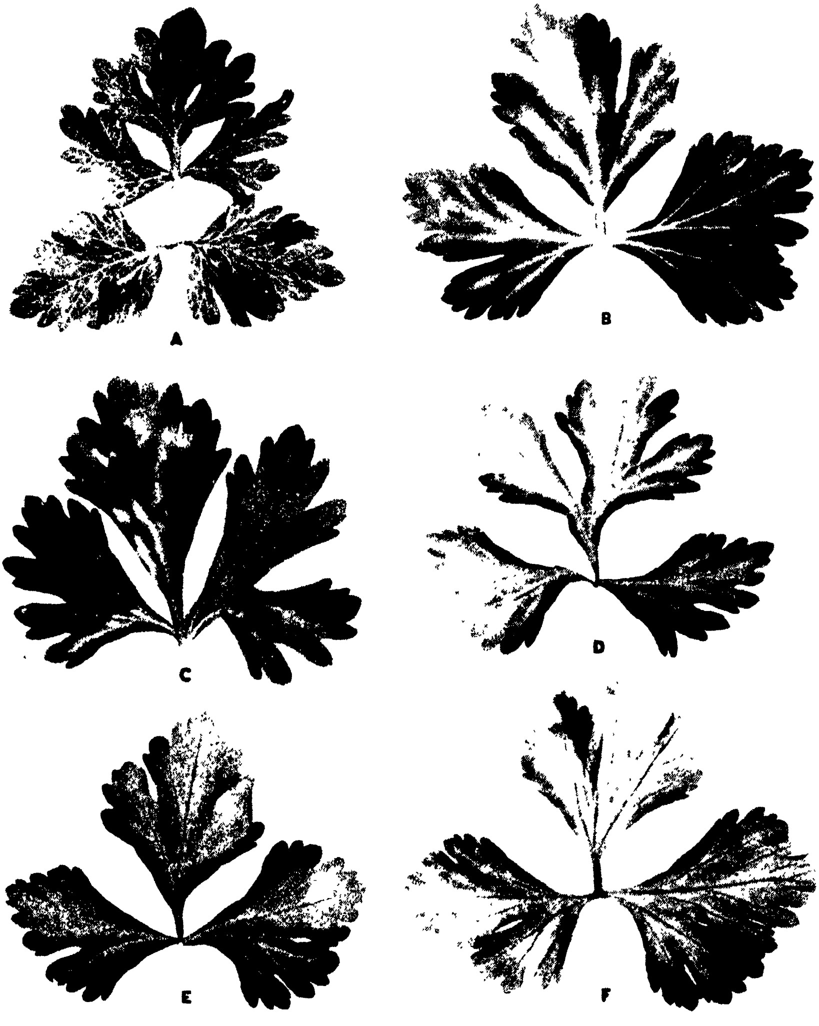

Plate 2.—Symptoms induced by the feeding of *Texananus spatulatus* on celery (*Apium graveolens dulce*) leaves: *A*, cleared veins and veinlets; *B*, yellow veinbanding and chlorosis; *C*, interveinal yellow areas; *D*, *E*, chlorosis spreading over most of the leaflets with yellow veinbanding still evident in chlorophyll areas; *F*, chlorosis of leaflet and necrosis of veins.

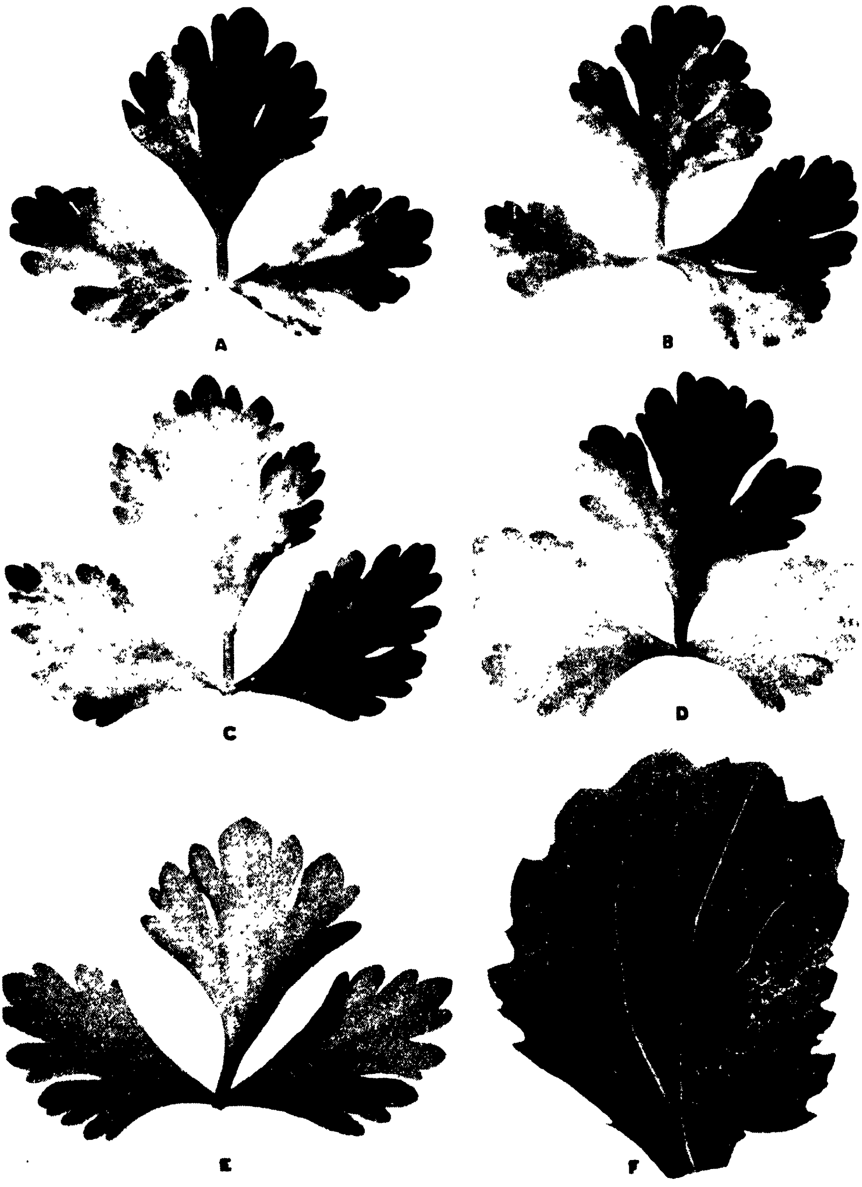

Plate 3.—Symptoms caused by the feeding of large populations of the mountain leafhopper, *Colladonus montanus*, on celery (*Apium graveolens dulce*) leaflets: *A*, *B*, mottling; *C*, *D*, *E*, chlorosis gradually spreading over the entire leaflets. *F*, cleared veins and veinlets on a portion of an outer China aster (*Callistephus chinensis*) leaf induced by a single adult *Acinopterus angulatus*.

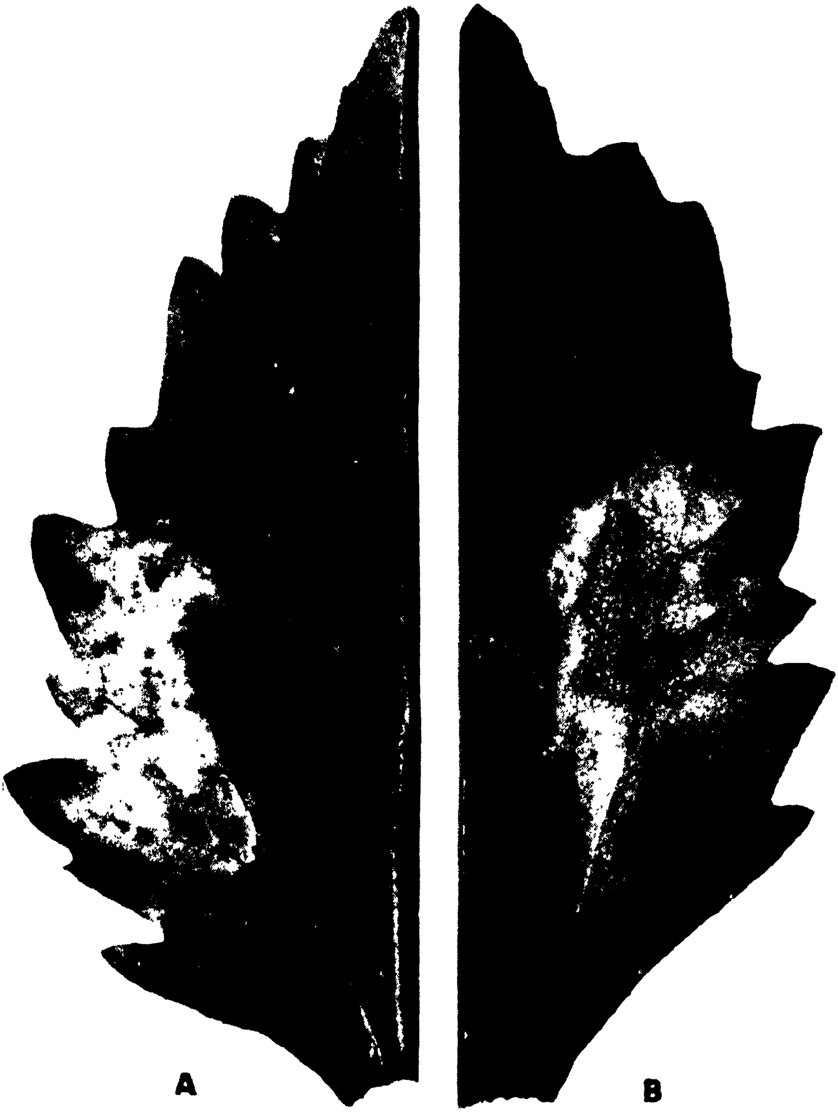

Plate 4.—Symptoms on China aster (*Callistephus chinensis*) leaves induced by the saliva of single nymphs of *Idiodonus heidemanni*. *A*, yellow discoloration of aster leaf serving as a protective resemblance with nymph of the same color; *B*, yellow discoloration and necrosis of aster leaf; the nymph was transferred from the yellow to the green portion of the leaf to contrast the color.

H I L G A R D I A

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DEPOSITIONAL AND WEATHER-RESISTING QUALITIES OF SOME COPPER FUNGICIDES AFFECTING THE CONTROL OF PEACH BLIGHT[1]

E. E. WILSON[2]

INTRODUCTION

IN THE TESTS reported in this paper, comparisons were made between the depositional and weather-resisting qualities of bordeaux and three so-called fixed copper fungicides used to control peach blight caused by the fungus *Coryneum beijerinckii* Oud. Each fixed copper preparation was combined with a supplement to increase the tenacity, or weather resistance, of the deposit.

STUDIES ON DEPOSITION

On Peach Twigs.—In field tests conducted for three seasons, water suspensions of basic copper sulfate A,[3] basic copper sulfate Z (containing zinc), cuprous oxide, and bordeaux mixture were applied in mid-November to peach trees after most of the leaves had fallen. One-half per cent of a cream-type oil emulsion, designated as supplement A,[4] was added to basic copper sulfate A. One and one-half per cent of an emulsive oil, supplement B, was added to basic copper sulfate Z and to cuprous oxide. The emulsifying agents and other ingredients of these supplements are unknown to the writer. Except in the first year of the tests, each preparation was applied to three randomized plots with an ordinary orchard sprayer operated at a pressure of 450 to 500 pounds per square inch.

In addition to these treatments, basic copper sulfate Z, suspended in an emulsive oil which was then emulsified in water (1 gallon of oil to 1 gallon of water), was applied by means of vapor-spraying equipment. This machine produces a finely divided mist by injecting the preparation into an air stream, which issues from ducts at the side of the machine.

After the sprays had dried, samples of twigs produced in the previous growing season were collected; the copper deposits were determined by the iodo-

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[3] The basic copper sulfate designated as A (Basi-Cop) contained 52 per cent metallic copper; that designated as Z (Zinc-Coposil) contained 19 per cent copper; the cuprous oxide (yellow coprocide) contained 84–85 per cent copper.

[4] The trade name of supplement A is Basi-Spred; of supplement B, Ortho-Adhesive.

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metric method as described in another paper (Wilson, 1942). In late February, other samples were taken and the residues remaining after weathering were determined. Table 1 gives these data, the results of four trials conducted in three different years.

Average initial deposits shown in the second column of table 1 differed considerably among the various preparations. This finding might be expected, since the copper content of the prepared sprays also differed. For example, preparations of basic copper sulfate Z, 6 pounds per 100 gallons of water, and cuprous oxide, 1½ pounds per 100 gallons, contain 0.137 and 0.152 per cent copper, respectively, as against 0.299 per cent for bordeaux of 10–10–100 strength. These preparations deposited, respectively, 19.6 and 24.7 milligrams of copper on each 100 grams of twigs, as against 32.8 milligrams to each 100 grams for bordeaux. When the initial deposits were compared with respect to the copper content of the spray preparation, however, all fixed copper materials proved to be deposited in greater relative amounts than bordeaux. The coefficients of deposit reported in figure 1 were computed as follows: The amount of copper deposit, y, of the material in question proportional to the deposit, b, of bordeaux (10–10–100) was obtained by $y = \frac{bx}{0.299}$, where x is the copper content (in per cent) of the preparation of the material in question

TABLE 1

COMPARISON OF COPPER FUNGICIDES WITH INITIAL DEPOSITS, RESISTANCE TO WEATHERING, AND CONTROL OF PEACH-TWIG INFECTION BY *Coryneum beijerinckii*, 1941–1944

Fungicide material, pounds per 100 gallons, and supplement used, expressed in per cent by volume	Milligrams of copper on 100 grams of twigs		Average coefficient of tenacity†	Average number of lesions on 100 twigs
	Average initial deposit	Average residue*		
Unsprayed	.	. .	.....	1,770
Bordeaux, 10-10-100	32.8	8.0	0.239	62
Bordeaux, 10-10-100; plus supplement B, 1 per cent	29.6	9.6	0.348	16
Bordeaux, 8-8-100; plus supplement B, 1 per cent	26.3	10.4	....	.....
Basic copper sulfate A, 5-100; plus supplement A, 0.5 per cent	47.2	9.8	0.207	99
Basic copper sulfate Z, 6-100; plus supplement B, 1.5 per cent	19.6	4.5	0.222	78
Basic copper sulfate Z, 8-100, plus supplement B, 1.5 per cent	26.9	4.3	.	..
Cuprous oxide, 1.5-100; plus supplement B, 1.5 per cent	24.7	5.6	0.239	186
Vapor application, basic copper sulfate Z, 75-100, in 50 gallons of an emulsive oil and 50 gallons of water, applied at the rate of 20 gallons per acre	19.4‡	6.7‡	0.345‡	28
Difference required for significance: 19:1 odds	9.6	3.6	0.135	.....
Difference required for significance: 99:1 odds	13.5	5.1	0.189	.
Calculated F value	10.2	5.1	1.61	....

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* After weathering for approximately 3.5 months; average rainfall, 8.59 inches.

† Tenacity coefficient = ratio $\frac{\text{residual spray deposit}}{\text{initial spray deposit}}$. These values, being the averages of the individual coefficients of tenacity, are not necessarily the same as those obtained by dividing the average residue by the average initial deposit.

‡ Excluded from analysis of variance.

and 0.299 is the per cent of copper in the bordeaux preparation. The observed deposit divided by the expected deposit gave the coefficient. The coefficient for bordeaux, 10–10–100, of course, would always be 1.0.

Bordeaux preparations with and without supplement B were deposited at the same relative level; but all fixed copper materials were deposited in amounts 29 to 49 per cent higher than bordeaux, 10–10–100, without supplement. A difference of 32 per cent is required for significance at 19:1 odds.

Since the amount of preparation applied per tree with the vapor equipment varied somewhat between tests and since each test of this treatment consisted of one plot only, the results given in table 1 were excluded from the statistical analyses. In these trials the vapor preparation gave deposits about equal to that of basic copper sulfate Z, 6–100, applied with the regular sprayer, and only 41 per cent lower than that of bordeaux, 10–10–100. On the other hand, deposits of basic copper sulfate Z applied by vapor equipment in a commercial almond orchard were 58 per cent lower than deposits of bordeaux, 10–10–100. Large variations in deposits by this method of application might arise from difference in the speed with which the sprayer is drawn past the trees. As will be shown later, the amount of spray retained by a solid surface up to the time the liquid begins to drain away is directly proportional to the length of the application period. Since the rate of liquid delivery from the vapor equipment is low, under ordinary circumstances the amount deposited on twig surfaces is seldom great enough to cause runoff. Thus, marked variations in the application period should result in differences in the amount of spray deposited on the surfaces; and since the vapor spray issues from more or less fixed outlets, the speed of the sprayer will determine the amount of deposit.

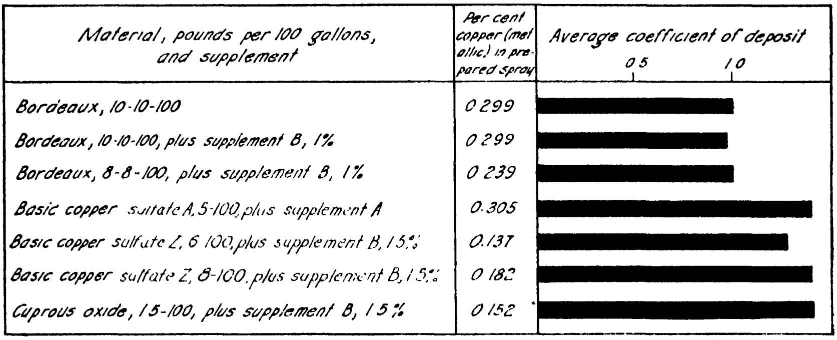

Fig. 1.—Deposition of copper fungicides on peach twigs relative to equivalent copper contents of the prepared sprays.

With the ordinary spray rig, on the other hand, the amount of liquid required to cover a given number of trees was approximately equal for all materials. Why, therefore, did the fixed copper materials consistently deposit relatively greater amounts of copper than did bordeaux? For a study of this point, the sprays had to be applied under conditions less variable than those in the orchard. A laboratory atomizing applicator, described earlier (Wilson, 1942), was employed. The surface to be sprayed was a thin film of cellulose nitrate deposited on glass slides.

On Artificial Surfaces.—To understand the method by which supplements might affect the spray deposits, one must consider how the liquid behaves during application. Hensill and Hoskins (1935) emphasized the dynamic nature of deposition. In the first place, the sprayed surfaces are seldom horizontal, so that gravity greatly affects the retention of liquid by the surface. Second, the forces that influence wetting and spreading properties are not permitted to attain equilibrium during application, because the deposited droplets are constantly being disturbed by the oncoming spray. Methods for determining the wetting and spreading under static conditions give results that are of little value for assessing the effects of these properties on retention of spray deposits. In general, however, if the surface is partially wetted by the preparation, an increase in the wetting properties will result in a decrease in deposit (Evans and Martin, 1935; Fajan and Martin, 1937; Hoskins and Ben-Amotz, 1938). Surfaces not wetted by a liquid will retain none of the deposit, because the droplets roll off. Under such conditions, an increase in the wetting properties increases the deposit (Hensill and Hoskins, 1935). Maximum retention of solutions and ordinary suspensions by a surface, therefore, occurs at some intermediate stage of wetting.

For solutions and ordinary suspensions, the maximum deposit is present when the liquid begins to run from the surface (Evans and Martin, 1935; Fajan and Martin, 1937). Continuing to spray beyond this stage ordinarily results in a decrease in the amount retained. Under certain conditions, however, an increase in deposit of a suspended solid occurs when application is continued beyond the stage at which the liquid runs off. Fajan and Martin (1938), for example, reported preferential retention of cuprous oxide by artificial surfaces when the preparation contained petroleum oil emulsified by Agral II. Such was not the case, however, with oil containing sulfite lye as an emulsifier. Under the microscope the oil droplets were seen to aggregate about the cuprous oxide particles when Agral II, but not when sulfite lye, was the emulsifier. Such observations indicate that a partial wetting of the cuprous oxide by the oil occurred with the Agral but not with the sulfite lye. A still more striking case of increased deposits resulting from application beyond the runoff stage is reported for the "dynamite" spray by Marshall (1937) and Marshall and Groves (1937). This spray is prepared by adding a petroleum oil containing one of the univalent soaps, such as ammonium or triethanolamine oleate, to a water suspension of lead arsenate. According to Marshall (1937), the lead arsenate becomes wetted by the oil before, or at the time the liquid comes in contact with the sprayed surface. Very high deposits of lead arsenate are obtained with this preparation.

Although the supplements used in the field trials herein reported contain oil, information on the nature of the emulsifying agents or other added materials was not available to the writer. When these supplements were added to water suspensions of the fixed copper fungicides, a distinct flocculation occurred. Under the microscope the flocculated phase was seen to consist of fungicide particles and water droplets surrounded by oil. Thus the system is apparently much like that of the "dynamite" spray described by Marshall (1937).

Aside from a slight curding, bordeaux was not affected by either supplements A or B, nor by a supplement prepared with oil and ammonium oleate. According to field tests, moreover, bordeaux deposition was not affected by supplement B. To show how supplements affect the deposition of the fixed copper fungicides and of bordeaux, the laboratory applicator was employed. Although the earlier work of this nature has been largely concerned with the amount of deposit at certain stages of spray application, such as "runoff"[5] (Evans and Martin, 1935; Fajan and Martin, 1937 and 1938), or after a definite amount of liquid had drained from the surface (Hensill and Hoskins, 1935; Hoskins and Ben-Amotz, 1938), more detailed data were desired in the present tests. In an attempt to determine the building up of the copper deposit before runoff and the status of that deposit after liquid had drained from the surface, glass slides coated with cellulose nitrate in series of five were sprayed for increasing lengths of time, and the amount of copper was ascertained for each spraying interval. In this way the amount of copper deposited by each fungicidal preparation could be compared at the same stage of application relative to runoff.

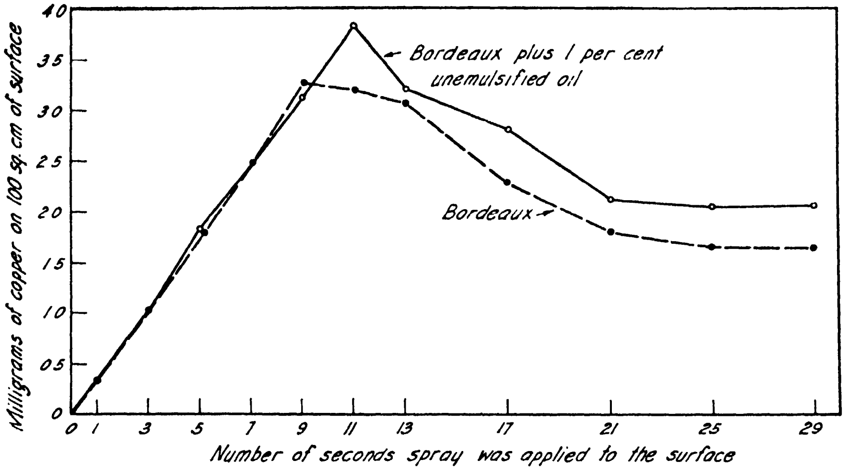

Fig. 2.—Deposition of 1 per cent bordeaux (by weight), and the same bordeaux plus 1 per cent of unemulsified petroleum oil, on cellulose nitrate surfaces.

The atomizer type of applicator has one main limitation: it will not deliver coarsely flocculated preparations at a constant rate, because the floccules tend to accumulate in the atomizer. Since this difficulty was encountered particularly with basic copper sulfate A combined with supplement A and with cuprous oxide combined with supplement B, bordeaux and basic copper sulfate Z with and without supplement B were used in the more detailed tests.

The first point to be examined is the deposition of bordeaux, which will illustrate what happened when an ordinary suspension or, for that matter, a solution was applied for increasing lengths of time. Figure 2 shows the results of tests in which series of slides were sprayed with 1 per cent bordeaux with and without unemulsified oil for 1, 3, 5, 7, 9, 11, 13, 17, 21, 25, and 29 seconds. Preliminary trials determined the length of time spraying must continue before runoff begins, and the time intervals were spaced so that one series of slides was sprayed just to the runoff stage. These slides, therefore, bore the maximum deposit.

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[5] "Runoff" is used for convenience in designating the stage when the liquid deposit begins to run from the surface, but before any is lost.

Before runoff started, deposition of bordeaux or bordeaux with an unemulsified oil was proportional to the length of time the spray was applied. The addition of 1 per cent unemulsified petroleum oil increased the length of time spraying was necessary to cause running of the liquid from the surface. Thus, when spraying was ended at the runoff stage, the deposit of copper was increased by the addition of unemulsified oil. This increased deposit had one or both of two causes: the oil decreased the wetting properties of the preparation, or the oil increased the viscosity of the preparation. The thickness of the liquid deposits of copper sulfate, bordeaux, and bordeaux plus an unemulsified oil sprayed to the runoff stage is illustrated by the photographs in figure 3. Here the liquid deposit of bordeaux is seen to be much thinner than that of bordeaux containing oil or that of copper sulfate. Apparently, therefore, bordeaux spray droplets spread over larger areas than those of the other preparations, probably because they wetted the surface to a greater degree.

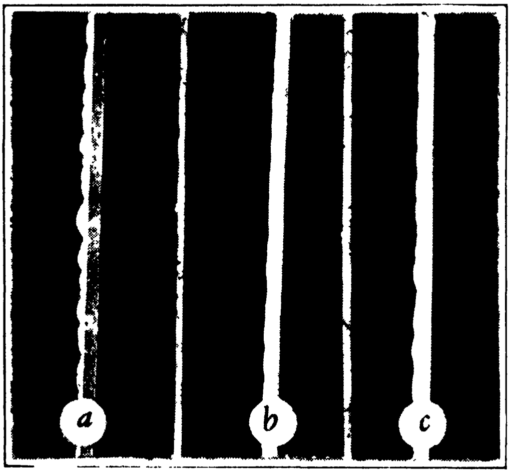

Fig. 3.—Edge view of glass slides showing the liquid deposits of various sprays at the stage just before the liquid began to run off: *a*, 1 per cent copper sulfate solution; *b*, 1 per cent bordeaux mixture; *c*, 1 per cent bordeaux mixture plus 1 per cent of an unemulsified petroleum oil. The spraying time necessary to start these sprays running down the surface was 13.4, 5.5, and 6.8 seconds, respectively.

Preparations of fixed copper fungicides without supplements wetted cellulose nitrate surfaces less than bordeaux. In consequence, when application ended at the runoff stage, these materials were deposited in greater amounts than bordeaux. On the whole, the supplements increased the amounts of these fungicides present when the runoff began. Although, for reasons given earlier, the results with cuprous oxide plus supplement B and basic sulfate A plus supplement A were not always reliable, the data given in figure 4 are believed to represent the situation fairly well.

In practice, spraying is seldom discontinued at the runoff stage. Dripping of spray from the surface occurs particularly on twigs at the periphery of the trees. From the artificial surface (fig. 2), bordeaux lost as much as 40 per cent of the deposit present at runoff when spraying was continued beyond that stage. It seemed desirable, therefore, to determine the behavior of the copper deposit during the time the spray was running from the surface. For this purpose, bordeaux and basic copper sulfate Z with and without supplements were applied to cellulose nitrate surfaces for 0, 4, 8, 12, 16, and 20 seconds after the liquid began to run off. The three supplements used were: first, the proprietary material designated B in the field tests; second, a petroleum oil (70 per cent U.R., 108 seconds Saybolt viscosity) containing 0.5 per cent ammonium oleate; third, the same petroleum oil emulsified with 6 milligrams of blood albumin per 100 cubic centimeters of oil. Both supplement B and the supplement containing ammonium oleate flocculated the fixed copper fungicide but did not affect bordeaux in this respect, whereas petroleum oil containing blood albumin did not flocculate either material.

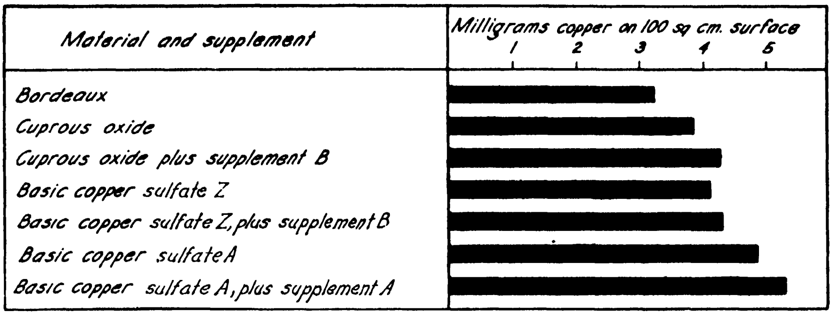

Fig. 4.—Deposition of bordeaux and of various other copper fungicides (with and without supplements) on cellulose nitrate surfaces sprayed to the runoff stage.

According to the results in figure 5, all supplements tended to lower somewhat the amount of bordeaux deposit retained by the surface at the time runoff began (0 seconds). As will be remembered, supplement B in the field tests (table 1) did not affect the deposit of bordeaux on peach twigs to a significant extent, though there appeared to be a very slight decrease. Either with or without supplements, the bordeaux deposit on cellulose nitrate decreased when spraying was continued beyond the runoff stage. In contrast, when no supplement or when oil containing blood albumin was added to the fixed copper, the deposit decreased when spraying was continued after runoff began; but when either supplement B or oil containing ammonium oleate was added, the curve was strikingly different. Deposits (curves *b* and *c* for basic copper sulfate Z) are seen to decrease as the liquid begins to run from the surface (4 to 8 seconds after runoff begins); but as application continues, they increase. Such behavior presumably results from the preferential retention of the copper phase. The liquid that runs off, consequently, contained less solid than when it struck the surface. Apparently, therefore, only certain types of emulsifying agents promote this phenomenon. Judging from the results with supplement A in the basic sulfate A and supplement B in cuprous oxide, preferential retention occurred here also; but detailed trials similar to those reported in figure 5 were not attempted, since the floccules of these materials clogged the atomizer, and thus reduced the accuracy of the results.

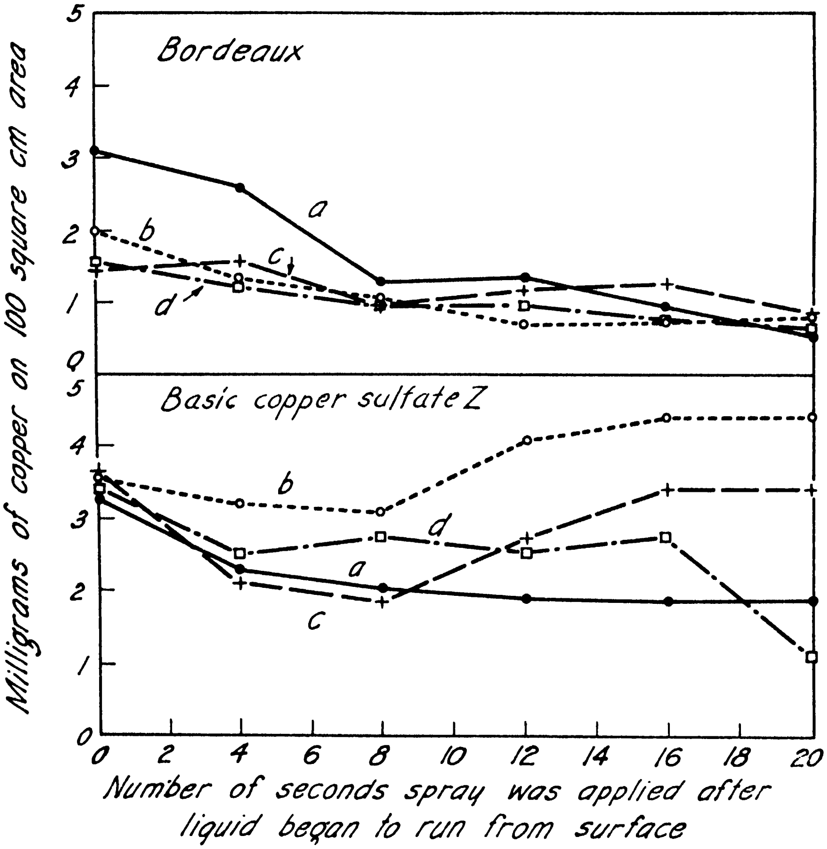

Fig. 5. -Effect of oil-containing supplements on the deposition of bordeaux and basic copper sulfate Z on cellulose nitrate surfaces: *a*, no supplement; *b*, 1.5 per cent supplement B; *c*, 1.5 per cent petroleum oil emulsified by ammonium oleate; *d*, 1.5 per cent petroleum oil emulsified by blood albumin.

WEATHER RESISTANCE

The standard program for preventing the peach-twig infection caused by *Coryneum beijerinckii* consists of a single spray applied in the fall after the leaves are off the trees. To remain effective against the disease, therefore, the fungicide must resist washing away by rains over a long period (as much as 100 days in some seasons).

Table 1, fourth column, shows the weather resistance (tenacity) of bordeaux with and without a supplement, and of the fixed copper fungicides with their respective supplements. The data for the vapor application were excluded from the analysis of variance because this material was applied to one plot only. On the whole, deposits by the vapor preparation resisted weatherings better than any of the other deposits. There was one exception: bordeaux with supplement B proved significantly more tenacious than basic copper sulfate A plus supplement A, or basic sulfate Z plus supplement B, and exhibited a strong tendency to resist weathering better than bordeaux without a supplement, or cuprous oxide plus supplement B.

CONTROL OF TWIG INFECTION

Twig infection, severe in 1942 and 1943, became too sparse for test purposes in 1944. According to the results in the fifth column of table 1, all materials reduced the disease greatly. Some differences in effectiveness, however, were apparent. For example, cuprous oxide appeared somewhat less effective than the other materials. On the whole, bordeaux (without supplement) gave better control than the fixed copper materials that were applied with the regular spray equipment. On the other hand, the vapor application of basic copper sulfate Z was, if anything, superior to bordeaux without supplement. Bordeaux containing supplement B (one year's tests), however, apparently surpassed all other materials.

TABLE 2

EFFECTIVENESS OF COPPER FUNGICIDES IN CONTROLLING PEACH-TWIG INFECTION CAUSED BY *Coryneum beijerinckii*, 1945

Fungicide material, pounds per 100 gallons, and supplement used, expressed in per cent by volume	Average number of lesions on 100 twigs
Unsprayed	388
Bordeaux, 10–10–100	45
Bordeaux, 8 8-100; plus supplement B, 1 per cent	26
Basic copper sulfate A, 5 100; plus supplement A, 0.5 per cent	47
Basic copper sulfate Z, 6–100; plus supplement B, 1.5 per cent	43
Cuprous oxide, 1.5–100; plus supplement B, 1.5 per cent	67

Table 2 gives additional data secured in 1945, when the disease was moderately severe. Again, bordeaux with supplement B proved most effective and cuprous oxide least effective, whereas the control by bordeaux without supplement, by basic copper sulfate A, and by basic copper sulfate Z were of the same order of effectiveness.

Table 1 shows a lack of correlation between the amount of copper residue remaining on the twigs throughout the winter and the prevention of twig infection. For example, the average residues of bordeaux, 10–10–100, plus supplement B and basic copper sulfate A differed but little; yet the bordeaux treatment was the more effective. Although the amount of copper was less on trees sprayed with basic copper sulfate Z than on trees sprayed with bordeaux 10–10–100, control of twig infection differed little between the two. Basic copper sulfate Z, as will be recalled, contains 19 per cent zinc, which possibly helped to prevent the disease.

SUMMARY AND CONCLUSIONS

Studies herein reported concerned orchard and laboratory comparisons of bordeaux and three fixed copper fungicides (plus proprietary adhesive) with respect to their depositional, weather-resisting, and disease-preventive qualities.

In proportion to the amount of copper in the spray preparations, the fixed copper materials deposited 29 to 49 per cent more copper than bordeaux did. On the other hand, various preparations of bordeaux, some with and some without an oil-type supplement, differed but little in this respect.

Laboratory tests were designed to study the deposition of the various preparations on an artificial surface. The materials were applied to glass slides (coated with cellulose nitrate) by means of a precision applicator for the same length of time, and the amount of copper retained by the surface was determined. The liquid deposit of bordeaux was retained by the surface in direct proportion to the length of the application period up to the time the liquid began to run from the vertically held surface. If application was continued beyond runoff, however, the deposit decreased for a time, but then reached a stage where it remained approximately constant. Sometimes the loss of copper through runoff was 40 per cent of the deposit present just before runoff began.

Addition of an unemulsified petroleum oil to bordeaux increased the amount of liquid deposit and, consequently, the amount of copper deposit retained by the surface at the runoff stage. The increased deposit was evidenced by the longer period of application necessary to cause the liquid to run from the surface, and was probably due to one or both of two factors: a decrease in the wetting properties of the spray preparation, or an increase in the viscosity of that preparation.

Water suspensions of the fixed copper fungicides wetted cellulose nitrate less readily than did bordeaux. For this reason the amount of liquid retained by vertical surfaces at the runoff stage was higher than when bordeaux was used. When application was continued beyond runoff, the liquid deposit of the suspensions decreased much as did that of bordeaux. When, however, certain proprietary supplements or a supplement containing a petroleum oil emulsified with ammonium oleate was added to these suspensions, copper was retained by the surface in increasing amounts as application continued beyond the runoff stage. This situation was attributed to the preferential retention of the oil, and the copper suspended therein, by the sprayed surface. According to microscopic evidence, the flocculated phase of these preparations consisted of oil globules enclosing fungicide particles and droplets of water. When petroleum oil emulsified with blood albumin was added to suspensions of fixed copper fungicides, no flocculation occurred; that is, the copper particles were not wetted by the oil. Preferential retention of these particles by the sprayed surface, moreover, did not occur with such preparations.

Under the experimental conditions, the solid phase of bordeaux was not wetted by the supplements; neither was there evidence of preferential retention.

Loss of deposit by weathering was determined in the orchard. In three seasons when rainfall averaged 8.59 inches, the coefficients of tenacity were as follows: bordeaux, 0.239; bordeaux with supplement B, 0.348; basic copper sulfate A (vapor application), 0.345; cuprous oxide, 0.239; basic copper sulfate Z (applied with regular spray rig), 0.222; basic copper sulfate A, 0.207. All fixed copper preparations were combined with an oil-type supplement.

Though all preparations greatly reduced twig infection by *Coryneum beijerinckii,* bordeaux with supplement B and the vapor application of basic copper sulfate Z appeared somewhat more effective than basic copper sulfate A, basic copper sulfate Z, and cuprous oxide.

The amount of copper residue remaining on the tree throughout the infection period (the winter months) was not always correlated with the degree of twig-infection control. This finding suggests that some residues may have been more toxic to fungus spores than others; but the data are insufficient for conclusions.

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SNAPDRAGON DOWNY MILDEW

C. E. YARWOOD

SNAPDRAGON DOWNY MILDEW[1]

C. E. YARWOOD[2]

SNAPDRAGON downy mildew, caused by *Peronospora antirrhini* Schroet., has been recorded only on *Antirrhinum orontium,* a wild species of Europe, and on *Antirrhinum majus,* the cultivated snapdragon. Kenneth F. Baker[3] found this mildew on *Antirrhinum nuttallianum* at Otay Lake, San Diego County, California, in 1941. All varieties of snapdragon inoculated by the writer, or observed under conditions of heavy natural infection, have been susceptible. Murphy (*13*)[4] indicates that dark-colored varieties are the most susceptible. In a heavy natural infection at Guadalupe, California, in 1940, breeding lines with dark, waxy foliage had fewer infected plants than light-colored ones.

Peronospora antirrhini was first reported on *Antirrhinum orontium* in 1874 (*15*) from Germany, on this same species in Switzerland in 1907 (*10*), and in Denmark in 1913 (*12*). It is not recorded to have caused commercial damage until 1936, when Murphy (*13*) in Ireland reported it to be severe on nursery seedlings of cultivated snapdragons. It has since been found in England (*1, 7, 8*), New South Wales (*2*), California (*9*), Pennsylvania (*11*), and Oklahoma (*14*). McWhorter[5] found the disease at Portland, Oregon, in the spring of 1944. Snapdragon mildew was first observed by the writer on May 25, 1938, on specimens brought in by a nurseryman near Hayward. By 1940 it not only was severe throughout the San Francisco Bay district, but also had appeared in southern California.

IMPORTANCE OF THE DISEASE

Snapdragon downy mildew has been principally a seedling disease of nursery plants. Systemically infected plants are unsalable. They are unsuitable for planting because they generally fail to grow and finally die. Losses have varied from none to all plants in a given planting. The standard practice in the San Francisco Bay area is to plant about 1,000 snapdragon seeds per flat, and later to transplant the resulting seedlings at the rate of 100 to 120 seedlings per flat. The plants are sold in the second flats within a few days to a few weeks after transplanting. If more than about 10 plants per flat show systemic infection, the entire flat is usually discarded. One nursery in Oakland discarded its entire stock of 600 flats of snapdragons at one time because of downy mildew infection. In 1940–1942 the infection was so generally destructive in southern California that it was impossible to obtain healthy seedlings. At a seed farm at Guadalupe, 90 to 95 per cent of the plants in 1,400 flats were lost because of mildew in January, 1940, and at a nursery in Los Angeles about 1,000 flats were discarded because of mildew in 1942. Murphy (*13*) and Green (*7, 8*) also indicate extensive nursery losses in Ireland and England.

Losses may also occur on greenhouse plants grown for cut flowers, but the writer has little information on how severe this may be.

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[3] All observations in southern California are those of Kenneth F. Baker, Associate Professor of Plant Pathology, and Associate Plant Pathologist in the Experiment Station.

[4] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

[5] F. P. McWhorter of Oregon State College, in a personal communication to the author dated December 10, 1945.

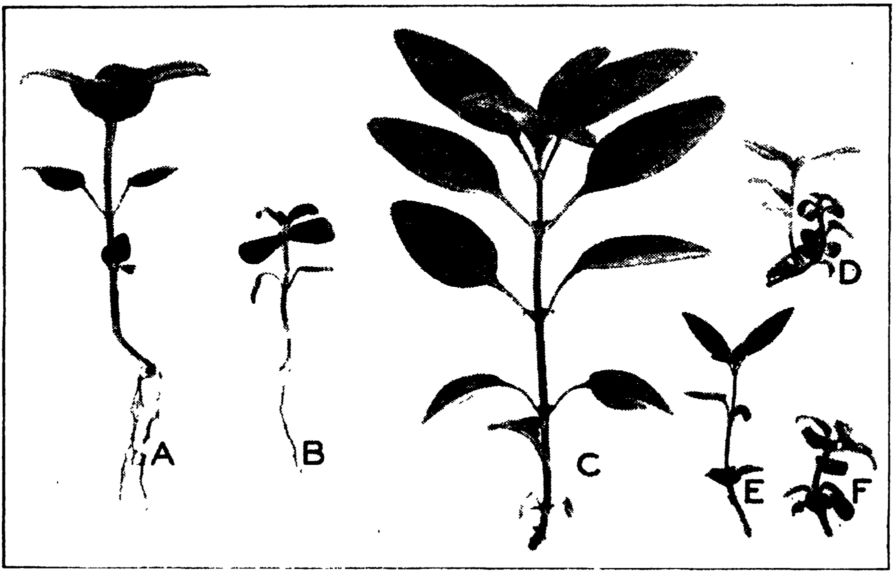

Fig. 1.—Symptoms of systematic infection of downy mildew on seedling snapdragons. *A*, healthy noninoculated plant; *B*, inoculated. *A* and *B* were the same age and size at time of inoculation on February 21, 1940; photographed March 7, fourteen days after inoculation. *C*, healthy noninoculated plant; *D*, *E*, *F*, inoculated; *C*, *D*, *E*, *F* were the same age and size at time of inoculation on February 1, 1940; *C*, *D*, *E*, *F*, photographed March 26, fifty-three days after inoculation; *D* shows recovery by growth of a lateral noninfected shoot; *E* shows recovery by growth of a terminal healthy shoot; and *F* shows no recovery.

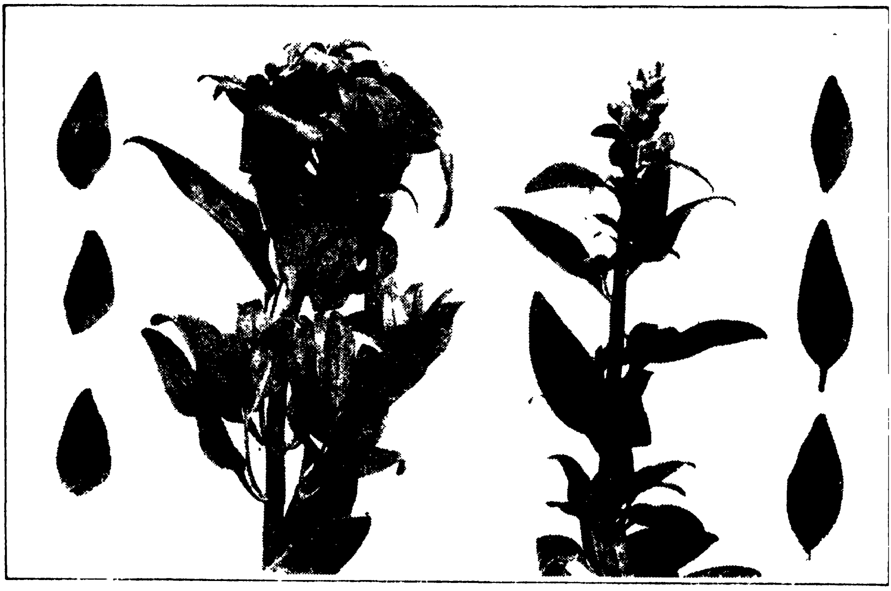

Fig. 2.—Symptoms of systemic infection of downy mildew on snapdragons grown in the greenhouse for cut flowers; plant and individual leaves on right are healthy; plant and individual leaves on left show systemic infection. Systemically infected plants are shorter than normal; leaves show basal infection. Photographed January 29, 1942.

SYMPTOMS

The most important infections of downy mildew on snapdragons are systemic and the symptoms are characteristic of systemic infections of other downy mildew diseases. On seedling plants there is a downward curling of the leaves, a reduction in the size of the leaves and of the plants, and the infected leaves are paler green than normal. On seedlings, downy mildew causes a stunting or killing which progresses from the top of the plant down to the soil surface; it is thus readily distinguished from the common damping-off of seedlings which kills the plants from the taproot in the soil up to the soil surface. Symptoms may appear from the time when the plant has two cotyledons and continue to appear until the plants are about 6 inches tall (fig. 1). They may appear again on the growing points of plants, 1 to 4 feet tall, which are coming into flower (fig. 2). On these large plants, there is a rosetting of the growing points. It is not suggested that plants between these specified stages of growth are immune, but infection has not been observed on them by the writer. Furthermore, in the writer's inoculation tests, systemic infection has not occurred on plants inoculated when they are more than about 4 inches tall. Unfavorable environmental factors may well have contributed to these failures. Local (nonsystemic) infections of leaves are common but are rarely destructive. They consist of pale rounded areas up to 15 millimeters in diameter with smooth diffuse margins.

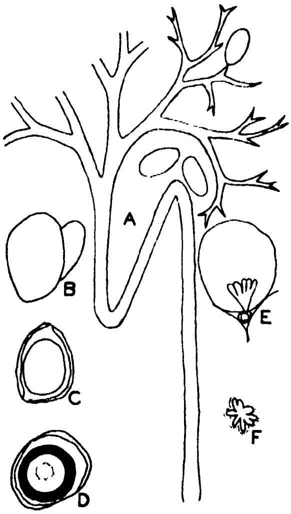

Fig. 3.—*Peronospora antirrhini: A,* portion of sporangiophore, showing attached and detached sporangia (the sporangiophore is illustrater as bent in order to reduce the size of the illustration); *B,* early stage in oöspore formation with antheridium on side of oögonium; *C,* later stage, oöspore formed; *D,* mature oöspore enclosed in oögonium; *E,* branched intracellular haustorium in host cell and intercellular hypha; and *F,* surface view of haustorium indicating protoplasmic sheath.

The disease is rare in garden or field plantings, but on May 24, C. M. Tompkins found the disease severe on seedling plants grown outdoors at Colma under conditions of overhead sprinkling irrigation but in a growing season without rain for several previous weeks.

ETIOLOGY

Snapdragon downy mildew is caused by *Peronospora antirrhini* Schroet.—a typical downy mildew. Sporangiophores and sporangia (conidiophores and conidia) are borne principally on the lower leaf surface, but also in abundance on the stems and upper surface of the leaves of young succulent plants. Sporangiophores emerge through the stomatal openings, are 350 to 704μ long, dichotomously branched, with 27 to 140 ultimate branches, each bearing an ovoid sporangium. Sporangia are 14–17 by 21–29μ and have been observed to germinate by a germ tube only. The internal intercellular mycelium bears intracellular haustoria with 4 to 8 fingerlike branches, similar to those of

Pseudoperonospora on cucumber and hop, and not seen previously on any member of the genus *Peronospora* observed by the writer. Oöspores 30 to 33μ in diameter in oögonia 43–52μ in diameter are produced in great abundance in the cortex and pith of systemically infected seedlings. Green (*8*) indicates that in England oöspores were very rare in living plants, but in the writer's material they have been more numerous than in any other downy mildew examined.

The morphologic features of *Peronospora antirrhini* are indicated in figure 3. Quantitative differences in the writer's description and illustrations from the descriptions and illustrations of other observers (*3, 6, 8, 14*) are not considered of any taxonomic significance. The branched haustoria are considered the most useful character in distinguishing this from most other species of *Peronospora.*

The shortest period in which symptoms have appeared in controlled tests has been 4 days after inoculation.

EPIDEMIOLOGY

Snapdragon downy mildew, like most other downy mildews, appears to be favored by low temperatures and high humidity. In one test, leaves from systemically infected plants placed in moist chambers and incubated at a range of temperatures failed to sporulate at 7° C and below, sporulated luxuriantly at 13° and moderately at 19° but not at 22° or above. In one test of the effect of temperature on spore germination, sporangia in drops of water on slides in petri dish moist chambers germinated as follows: at 1°, 0; 4°, 0; 7°, 11 per cent; 10°, 65 per cent; 13°, 37 per cent; 16°, 26 per cent; 19°, 0; and at 22°, 0. No tests of the effect of temperature on infection under controlled conditions have been performed, but greenhouse inoculations of plants during the winter and early spring months have been successful while inoculations during the hotter summer months have been distinctly less so. On the basis of the limited information available, the writer believes the optimum temperature for snapdragon downy mildew is about 10° C.

In the San Francisco Bay region, snapdragon seedlings are grown principally from February to May in unheated greenhouses as bedding plants for sale, and it is under these conditions that downy mildew has been so destructive. A maximum-minimum thermometer placed in one such greenhouse from February 24 to April 2, 1940, and reset every day showed a minimum temperature ranging from 40° to 50° F (4° to 10° C) and averaged 44° F (7° C); while the maximum temperature ranged from 61° to 95° F (16° to 35° C) and averaged 74° F (23° C). The luxuriant development and active spread of downy mildew in greenhouse culture, more active according to the writer's observation than in outdoor culture, indicates that rain plays little necessary part in the epidemiology of this disease.

In southern California, on the other hand, snapdragon seedlings are grown principally under lath or cloth, or outdoors, and the disease is severe under these conditions. Epidemics there are associated with dark, foggy, humid, and rainy weather.

Environmental conditions during the incubation period (from inoculation until the pathogen establishes nutritive relations with the host) are undoubtedly most critical in determining the amount of infection. Dry seedling plants in pots were dusted with dry sporangia and placed in a variety of environments for 24 hours and then returned to a heated greenhouse where the amount of systemic infection was recorded 15 days after inoculation. No water was applied to the leaves in any case. The percentages of systemic infection in four trials with inoculations in varying environments, made January 21–28, 1942, with an average of 9 plants per treatment in each trial were as follows:

	Per cent
Moist chamber in heated greenhouse, about 20°C	54
Open bench in heated greenhouse, about 20°C	7
Moist chamber in unheated greenhouse, 7°–15°C	100
Open bench in unheated greenhouse, 7°–15°C	52
Open bench in unheated greenhouse, and with electric fan directed on plants to reduce humidity, 7°–15°C	0

Environmental conditions following the incubation period are probably less important than those during incubation. Plants which had been inoculated and held in a moist chamber overnight were placed in the following contrasting environments: a heated greenhouse with the temperature about 20° C, natural outdoor environment, an unheated greenhouse, and a lathhouse. In two tests, with an average of 12 plants in each environment in each test, the average percentages of systemic infection in the environments listed were 47, 24, 74, and 40 respectively—least, therefore, in the plants left out of doors.

The writer believes that considerable infection in commercial greenhouses is traceable to the contamination of healthy plants in the process of transplanting. Workers contaminate their hands with sporangia by handling infected plants which may or may not show distinct symptoms, and unknowingly transfer these sporangia to healthy plants. On two occasions heavy infection appeared in flats about a week after transplanting.

CONTROL BY SANITATION AND VENTILATION

As snapdragon downy mildew does not yet occur in most regions, its control in these regions by exclusion would appear in order. The most likely method by which the disease could move long distances would appear to be by oöspores in the seed. The sequence of occurrence in Ireland in 1936 (*13*), England in 1937 (*1*), California in 1938 (*9*), New South Wales in 1941 (*2*), Oregon in 1944, Pennsylvania in 1945 (*11*), and Oklahoma in 1945 (*14*) would seem to depend on some such mode of primary distribution. Since oöspores from seedling infections would seem to have little chance of getting into the seed, whereas oöspores in terminal infections of flowering plants could easily contaminate the seed, it would appear that this latter type of infection may be more common than recorded. In a disease-free district it would seem to be a wise precaution not to use seed from diseased areas, if this is possible. Within regions where the disease already occurs, local exclusion, such as avoiding carrying infectious material from an infested to a noninfested nursery, would appear worth while.

Oöspores in infected seedlings which die and disintegrate can readily contaminate the soil in which they are grown, and it would appear logical to expect that this contaminated soil might be an important source of primary infection. Attempts to eradicate the disease by destroying infected plants are reported by Murphy (*13*), Green (*8*), and Harris (*9*). Those by the latter two were apparently unsuccessful. Two nurserymen have told the writer of their attempts to control the disease by disposing of all snapdragon plants, but the disease reappeared next year. On the other hand, at one seed farm at Guadalupe the sterilization of the soil used for snapdragon seedlings was associated with reduced downy mildew infection for two years. Some nurseries have escaped the disease without any specific precautionary measures.

Where the disease occurs in greenhouses, control by manipulation of environment would seem a definite possibility. Practices which reduce the humidity at the plant surfaces should reduce infection. During the course of greenhouse studies of the disease for two seasons, the writer's plants grown in a heated greenhouse never became infected naturally, though infected plants were frequently close to them. When such plants were heavily dusted with spores, however, 3 out of 16 plants became infected in one test but none became infected in three other tests. Exposing inoculated plants to an air current from an electric fan entirely prevented infection of plants in an unheated greenhouse, though similar plants not exposed to the fan showed 52 per cent infection. This method of control by air movement would appear practical.

CONTROL WITH FUNGICIDES

The success with paradichlorobenzene for the control of tobacco downy mildew (*5*) suggested its use for snapdragon downy mildew. To treat with paradichlorobenzene, frames 23 × 14 × 3 inches (the same size as the flats to be treated) and covered with two layers of cheesecloth on the upper side, were placed over the test flats of plants, and the desired amount of medium paradichlorobenzene crystals was scattered over the surface of the cheesecloth. The entire flats and frames were then covered tightly with oilcloth to confine the fumes overnight, and the oilcloth and frame removed in the morning. Dosages ranged from 0.5 to 2.5 grams paradichlorobenzene per flat and this treatment was repeated every second or third night. Treatments were applied at 5 to 6 p.m. and the treated flats were uncovered about 8 a.m. next morning. In all instances flats of heavily infected plants were placed among the test flats to insure an adequate amount of inoculum but the plants were not artificially inoculated. The results of four tests at two nurseries, presented in table 1, indicate effective control, but with crop injury at dosages of 2 grams or more per flat. Even in the absence of localized necrotic injury this treatment stunted the plants somewhat and on warm nights severe injury sometimes resulted. It is likely that nightly applications with the lower dosages would have been more successful but this type of treatment was not considered practical by either nurserymen or the writer.

Hydrogen sulfide diluted with air or vapors from dilute lime-sulfur was relatively ineffective in eradicating snapdragon downy mildew (*16*), and these vapor mixtures could be used to kill rust infections without killing the downy mildew in the same plants (*17*).

Spray and dust treatments appear more adaptable to nursery practice than vapor treatments, and a variety of sprays and dusts were tried. In some tests healthy plants were treated with the test fungicides and artificially inoculated within 24 hours. Rosin lime-sulfur (*19*) and cuprous oxide cottonseed oil were tested more thoroughly than other sprays. Rosin lime-sulfur was prepared by adding the required amount of rosin soap and then the required amount of concentrated lime-sulfur to the required amount of water. For example, to prepare 100 cc of 0.5 per cent rosin lime-sulfur, 99 cc of water was first added to a container, then 0.5 cc of rosin soap, and then 0.5 cc lime-sulfur and the whole agitated. Rosin soap was prepared by heating together 14 parts by weight of water, 5 parts of rosin, and 1 part of potassium hydroxide. Results of four trials with a series of dosages of rosin lime-sulfur and cuprous oxide cottonseed oil were analyzed by dosage response methods (*4*).

TABLE 1

CONTROL OF SNAPDRAGON DOWNY MILDEW IN COMMERCIAL NURSERIES WITH PARADICHLOROBENZENE CRYSTALS

Grams of paradichlorobenzene at each treatment	Number of plants counted	Per cent of plants showing systemic infection	Plant injury from paradichlorobenzene
0.0.	1,355	53.0	None
0.5...	73	22.0	None
1.0..	376	22.0	None
1.5.	374	18.0	Trace
2.0......	444	0.7	Moderate
2.5. ..	176	1 5	Severe

The dosage for 95 per cent control was about 0.3 per cent rosin soap plus 0.3 per cent lime-sulfur for the first mixture and 0.17 per cent cuprous oxide plus 0.17 per cent self-emulsifying cottonseed oil for the second mixture. The curve slopes (mortality probits per log. dose) as determined for data showing between 50 and 100 per cent control was 2.9 for the rosin lime-sulfur and 3.5 for the cuprous oxide mixture. The same and higher concentrations of lime-sulfur and cuprous oxide without the rosin soap or cottonseed oil supplements were less effective. Other spray mixtures tried, and the corresponding percentages of infection resulting, were as follows: 1 per cent bordeaux, 25 per cent; 0.2 per cent bordeaux plus 0.05 per cent glyceryl alkyl resin, 0; 1 per cent burgundy, 0; 0.1 per cent copper sulfate plus 1 per cent rosin soap, 0; 2 per cent of a proprietary copper ammonium carbonate solution containing 2 per cent copper, 25; and 0.03 per cent ferric dimethyl dithiocarbonate (Fermate) plus 0.05 per cent glyceryl alkyl resin, 0.

If effective, dusts are more acceptable to growers than sprays. In tests of methods of evaluating sulfur dust for the control of five downy mildew diseases, sulfur was more consistently effective against snapdragon mildew than against the downy mildews of hop, cucumber, onion, or lettuce (*18*). In six trials in which snapdragon infection in the controls averaged 86 per cent, sulfur dust entirely prevented infection in three trials, but 22, 65, and 100 per cent infection occurred on sulfured plants in three other trials. The cause of these discrepancies is not known, but it may be related to the washing off of the sulfur in the normal practice of watering. Experiment has clearly shown that sulfur dust applied to snapdragons can be so effectively removed by washing the plants with a water spray that the sulfured washed plants can be readily infected with downy mildew or rust. Other dusts tested and the percentage of infection associated with their use were: pure cuprous oxide, 0; copper lime dust, 30 per cent; a dust consisting of 10 per cent cuprous oxide plus 10 per cent iron oxide plus 80 per cent filler, 40 per cent; and a dust consisting of 50 per cent sulfur plus 5 per cent basic copper sulfate plus 10 per cent iron oxide plus 35 per cent filler, 43 per cent.

The spray (rosin lime-sulfur) and dust (sulfur) which appeared most promising at the time were compared in a test in which different groups of plants were treated separately with the test fungicides at intervals of three days (all being treated the first day of the test), appropriate checks were maintained, and all were exposed to conditions of heavy natural inoculation in a cool unheated greenhouse. The test was started December 27, 1940, and results were recorded January 22, 1941. The checks showed 92 per cent infection, the rosin lime-sulfur series showed 0, 2, 7, and 15 per cent infection on plants treated every 3, 6, 9, and 12 days, respectively, and the sulfur-dust series showed 59, 32, 27, and 39 per cent infection in a similar series. It is obvious that the spray was much more pronounced in its protective action than the dust.

TABLE 2

CONTROL OF SNAPDRAGON DOWNY MILDEW IN COMMERCIAL NURSERIES WITH SPRAYS AND DUSTS

Treatment	Per cent infection		
	Applications every 8 days, Nov. 21 to Jan. 24	Applications every 3 days, April 9 to 25	Applications every 3 days, April 12 to May 3
Control	80	80	59
0.2 per cent cuprous oxide plus 0.2 per cent emulsified cottonseed oil	..	29	3
0.2 per cent bordeaux plus 0.05 per cent glyceryl alkyl resin	13	..	..
0.5 per cent rosin soap plus 0.5 per cent lime-sulfur	11	26	0.5
Sulfur dust	76	4	1.0
Dust consisting of 10 per cent cuprous oxide, 10 per cent iron oxide, and 80 per cent filler	..	55	11
Dust consisting of 50 per cent sulfur, 5 per cent basic copper sulfate, 10 per cent iron oxide, and 35 per cent filler	32	..	..

In addition to the spray and dust trials in the experimental greenhouses, trials of sprays and dusts were made in commercial nurseries. As sources of inoculum, flats of heavily infected plants were maintained close to the test flats. Treatments were applied every 3 to 8 days, starting when the seedlings were coming through the soil or about that time. Results of three typical tests are given in table 2. In these, as in other tests of this type not reported, no treatment ever gave perfect control though with most treatments disease control was marked, and the results might be considered commercially satisfactory. Rosin lime-sulfur spray, with an average of 82 per cent control, was perhaps the best treatment; but sulfur dust at 3-day intervals was a close rival.

CONTROL BY RESISTANT VARIETIES

One seed company in southern California attempted to develop mildew-resistant snapdragons but the attempt was abandoned in favor of fungicidal control with rosin lime-sulfur. The possibility that seed produced by plants which recovered from systemic infection (fig. 1) might produce seedlings carrying factors for resistance was investigated. Seed was saved from 2 plants which had recovered by sending out healthy shoots from the systemically infected growing point. In the first trial, 27 seedlings from such seed were inoculated and all became systemically infected. This line of investigation was therefore discontinued.

SUMMARY

Snapdragon downy mildew, a relatively new disease of restricted world distribution, has been severe on nursery seedlings in the San Francisco Bay area since 1938, and in southern California since 1939. Only *Antirrhinum orontium, A. majus,* and *A. nuttallianum* are known to be affected.

In a suitable environment, symptoms may appear in as little as 4 days after inoculation. Symptoms of systemic infection consist of a down curling, paling, and rosetting of the leaves and a stunting of the plant. Local infections, which are of little importance, cause the formation of pale-green areas with diffuse margins. Recovery from systemic infection is frequently manifested by the formation of noninfected shoots from systemically infected plants. On plants about to come into flower a rosetting of the growing point occurs.

The causal organism *Peronospora antirrhini* has dichotomously branched sporangiophores and branched haustoria and produces oöspores in abundance in the cortex of systemically infected plants. Germination of the sporangia is by a germ tube. The optimum temperature for the causal organism is about 10° C. High humidity is necessary for infection.

Control by forced air circulation, by the vapor from paradichlorobenzene crystals, by sulfur dust, and by rosin lime-sulfur and other sprays has been demonstrated. The first and last of these treatments are considered most practical in localities where the disease has become established.

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H I L G A R D I A

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A STUDY OF TRANZSCHELIA PRUNI-SPINOSAE ON PRUNUS SPECIES IN CALIFORNIA[1, 2]

CLAYTON O. SMITH[3]

INTRODUCTION

THE GENUS *Prunus* comprises a heterogeneous collection of plants originating in many different parts of the world. The foliage of these plants is at times attacked by plant rust caused by various species of *Tranzschelia.*

The plant-rust genus *Tranzschelia,* as established by Arthur(*1*)[4] in 1906, consisted of a number of macrocyclic and microcyclic species, one of the macrocyclic species being the heteroecious rust of *Prunus* spp., *T. pruni-spinosae* (Pers.) Diet., formerly described as *Puccinia pruni-spinosae* Pers., but now separated from *Puccinia* because of differences in morphological characters.

This rust of *Prunus* is world wide in its distribution and probably occurs wherever *Prunus* species are grown. It is widely distributed in Europe and occurs in Asia (China, India, and Japan), Africa (Egypt and Uganda), Australia, and New Zealand. In South America this rust is found where *Prunus* is indigenous, in the mountains of Brazil, Colombia, Uruguay, and Venezuela. It is also reported from Mexico and Central America. In the United States it is found in the eastern states and is especially prevalent in the southeastern states and along the Pacific coast.

Other species of *Tranzschelia* found on the foliage of *Prunus* spp. and described by Tranzschel and Litvinov (*11*) should be listed: *T. japonica* on *P. armeniaca* var. *Ansu* and on *P. mume; T. microcerasi* on *P. microcarpa* and on six other species of *Prunus,* all from Central Asia; and *T. arthurii* on *P. serotina,* in the United States "(Michigan, leg. C. H. Hicks; Iowa, leg. Holway)."

The present study concerns observations of the rust *Tranzschelia pruni-spinosae,* both natural infection and that induced by artificial inoculation, on different species of *Prunus* in California. The study has extended over a series of years, though many of the artificial inoculations were made during the years 1942 and 1943.

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[1] Received for publication July 2, 1946.

[2] Paper No. 548, University of California Citrus Experiment Station, Riverside, California.

[3] Associate in the Experiment Station, Emeritus.

[4] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

IMPORTANCE AND PERSISTENCE OF RUST ON PRUNUS SPECIES IN CALIFORNIA

The rust fungus *Tranzschelia pruni-spinosae* on *Prunus* spp. in California is variable in its severity from year to year, probably largely as a result of meteorological conditions, though isolated trees have been observed to be attacked year after year, even in years when rust was not especially prevalent.

Infection late in the season, near time of leaf fall, is not regarded as of much economic importance, although the rust may be abundant on the leaves at this time and some hastening of leaf fall may result. But an infection early in the season (April or May) may spread under favorable conditions and cause premature defoliation in the summer and early autumn (July to September) and serious injury to the tree. Such defoliation may be especially severe on trees that hold infected, living leaves into the dormant period.

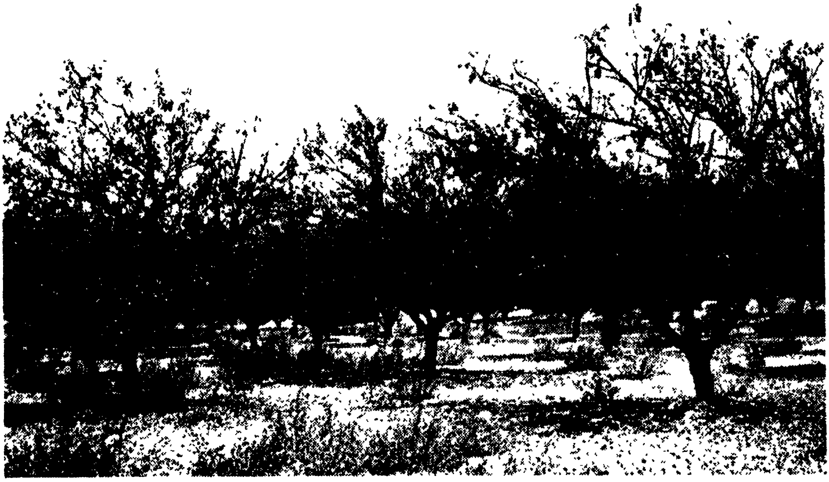

Fig. 1.—An apricot orchard infected with rust, *Tranzschelia pruni-spinosae*. Trees were nearly defoliated in July. (Photographed by J. T. Barrett.)

Goldsworthy and Smith (*6*) report an epidemic of rust that caused defoliation on cling peaches. Barrett (*2*) describes a defoliation of apricots (fig. 1) in southern California, caused by rust that overwintered and served as a source of inoculum for new shoot growth stimulated by early fall pruning. Rust is also severe at times on nursery stock.

It is not altogether certain how the inoculum is carried over the dormant season, but various ways have been suggested. Goldsworthy and Smith (*6*) found that one-year-old twigs of peach infected in the autumn formed pustules of urediospores the following spring. Dunegan and Smith (*5*) have shown that low temperatures greatly increase the longevity of the urediospores. It is possible, therefore, that the urediospores may live over winter on the leaves, especially on living leaves infected with rust.

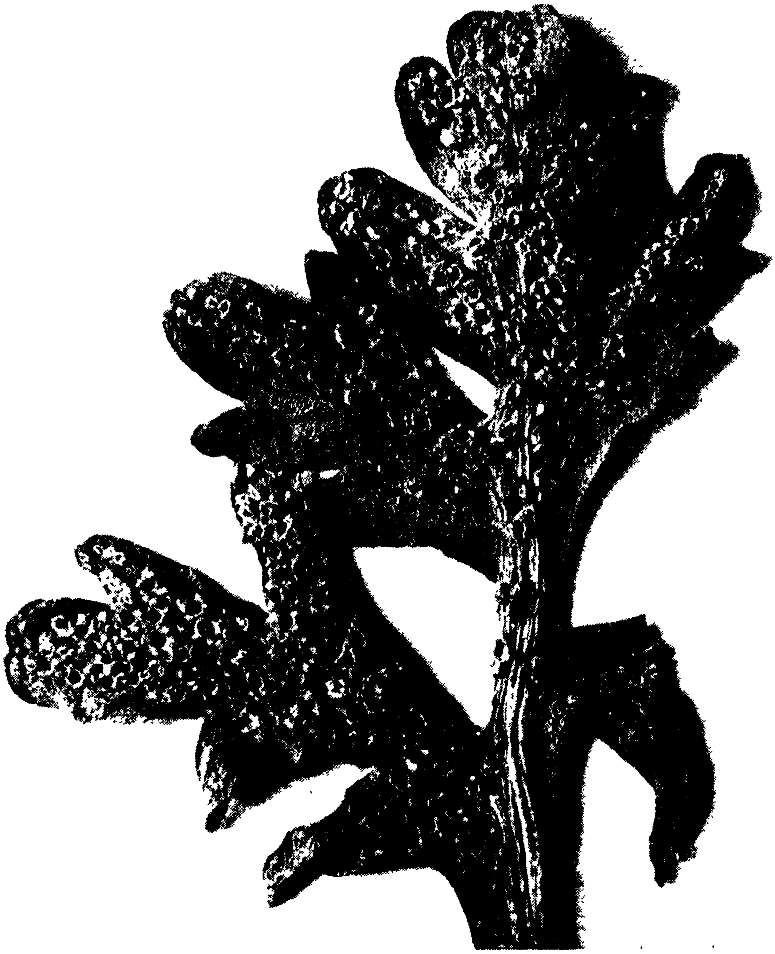

Fig. 2.—*Aecidium punctatum,* the alternate stage of the rust *Tranzschelia pruni-spinosae,* on *Anemone coronaria.* (Natural size.) (Collected by H. S. Fawcett in 1916. Photographed by J. T. Barrett.)

DIFFERENT RUST FORMS—THE TYPICA AND DISCOLOR TYPES AND THE CLUSTER-CUP OR AECIDIAL STAGE

Dunegan (*4*) has shown that there are two types of the rust *Tranzschelia pruni-spinosae* on *Prunus,* distinguished by the characteristics of the teliospores. In the typica type, the apical and basal cells of the two-celled teliospores are approximately the same shape, size, and color, and are uniformly coarsely verrucose over the entire surface. In the discolor type (regarded by some as *Tranzschelia discolor* Fuckel), the apical cell is globoid and coarsely verrucose over the entire surface, but the basal cell varies in shape from globoid to irregular, and its walls are never completely verrucose, in some specimens appearing almost entirely smooth.

Dunegan and Smith (*5*) describe differences in the germination of typica and discolor types of teliospores. The teliospores of typica type germinate near the apex of the apical cell and near the pedicel of the basal cell. In the discolor type, the germ tubes are produced near the septum.

The typica type of rust is found for the most part on the native or wild species of *Prunus*. The discolor type occurs almost entirely on cultivated species of *Prunus* (peach, apricot, almond, and the plum *P. domestica*), although Dunegan (*4*) has observed that certain native species of *Prunus* (*P. hortulana, P. mexicana,* and *P. besseyi*) are also susceptible.

The alternate or cluster-cup stage (*Aecidium punctatum*) of *Tranzschelia pruni-spinosae,* which develops on *Anemone* (fig. 2), has been found and studied in California by Scott and Stout (*7*), who observed that aecial spores were pathogenic on such cultivated species of *Prunus* as peach, almond, plum, and prune, but not on cherry. This stage apparently occurs rarely in California and is of limited importance. *Anemone coronaria,* the alternate host for rust of the discolor type, is planted in gardens in California but is not indigenous as it is in Europe, where it is apparently of considerable importance in carrying the rust over the dormant period of the *Prunus* spp. The indigenous species of *Anemone* and *Thalictrum* in California are for the most part mountain species and probably would be of limited importance as hosts if they should prove to be susceptible. In the United States, different species of *Anemone, Hepatica, Thalictrum,* and *Ranunculus* can be attacked by the cluster-cup stage.

METHODS OF INVESTIGATION

Observations and collection of rust material have been made over a series of years, in various parts of California, and notes have been taken on the presence or absence of rust on the different species of *Prunus* growing in mixed plantings and exposed to an abundance of inoculum from nearby infected peaches and plums (*P. domestica*). These observations have been made (1) in an experimental nursery at Riverside, where only a few of the peach rootstocks (Lovell seedlings) were successfully budded to other species of *Prunus,* and where the peach stock had sprouted and was severely infected with rust; (2) in experimental plots consisting of different species of *Prunus* growing in an irregular order of planting, located at Riverside and at Whittier, California, in the earlier years of this investigation; (3) in mixed experimental plantings at Moreno and Beaumont, California; and (4) in controlled experiments at Riverside, in which artificial inoculations of leaves on the more important species of *Prunus* were made to determine whether differences in pathogenicity would indicate distinct strains of rust, as reported by Thomas, Gilmer, and Scott (*10*) and suggested by field observations.

OBSERVATIONS OF RUST INFECTION IN AN EXPERIMENTAL NURSERY AND IN ORCHARD PLOTS

Observations of rust infection at Riverside, in an experimental nursery consisting of different species and varieties of *Prunus* budded on peach stock, extended over a two-year period, 1936–1937, inclusive. (The third year, 1938, showed so light a rust infection in this nursery that no data were available.)

The peach stocks were only in part budded to the other sorts, and were allowed to grow and serve as sources of inoculum, since they were severely infected with rust. All the trees, therefore, including those that escaped infection, must have been dusted with the rust spores at various times during the season. Rust infection in this nursery is summarized in table 1. Some of the hosts were severely infected in each of the two years; others of the host species were free from rust in one or both of the years.

TABLE 1

NATURAL RUST INFECTION OF *Prunus* SPP. IN AN EXPERIMENTAL NURSERY

Host species and source	December 12, 1936		October 8, 1937	
	Leaf infection*	Defoliation	Leaf infection*	Defoliation
Prunus amygdalus, Ne Plus Ultra almond	+	Slight	+	Slight
P. amygdalus, Nonpareil almond	+T	Medium	.	.
P. armeniaca, Royal apricot	+T	Medium	+	Slight
P. angustifolia var. *Watsonii*, seedlings	None	None	None	None
P. bokhariensis, P. I. 40224	None	None	None	None
P. cerasifera, from Arnold Arboretum	+T	Medium	+T	Medium
P. davidiana, seedling			+	Slight
P. davidiana, P.I. 36664	+	Medium	+T	Slight
P. hortulana, from Arnold Arboretum	?	None	+	None
P. mume, P.I. 47950	+	Medium	+T	Slight
P. munsoniana, from Arnold Arboretum		..	None	None
P. persica, seedling	+	Severe	+	Severe
P. salicina, Satsuma plum	+	None	Flecks	None
P. salicina, Wickson plum		.	+	None
P. tangutica, P.I. 40010	+	Severe	+	Severe
P. tomentosa, P.I. 36086	None	None	None	None
P. umbellata, seedling	+	Medium	+	Slight

* + = positive results indicated by leaf spot, uredia, and, usually, urediospore.
T = teliospore of discolor type present.

Observations of rust infection over a three-year period, 1936, 1937, and 1939, in orchard plots at Moreno and Beaumont, indicated an erratic occurrence of rust on the various species of *Prunus*. In both plots there was a miscellaneous collection of species and varieties of *Prunus*. Rust in the Moreno plot was largely on peach, however, and the intermingling of the rust-covered foliage of the peach with that of the other species afforded favorable conditions for infection. In the Beaumont plot rust was severe on almond all three years. The rust, in 1939, first appeared on almond trees that had been slightly rusted the previous year, and rapidly became severe, with defoliation beginning in July and continuing during the remainder of the year. Rust was well distributed over the almond planting early in the season, but was rare on peach until late in the season. Some of the almonds were topworked to varieties of *Prunus domestica* (French and Standard prunes) and *P. salicina* (Santa Rosa, Satsuma, and Wickson plums), the intermingling of the rust-covered foliage of the almond with that of the other species thus affording favorable conditions for infection.

Rust was observed in both plots on *Prunus armeniaca, P. amygdalus* (Ne Plus Ultra, Texas, Nonpareil, and I.X.L. varieties), *P. persica* (J. H. Hale, Lovell, Babcock, Krummel, Elberta, and C. O. Smith varieties), and on *P. mahaleb* (mahaleb cherry). In the Moreno plot, rust was also observed on *P. besseyi, P. cerasifera* var. *Pissardii, P. davidiana, P. emarginata,* and *P. mume.* Rust was not observed in either the Moreno or the Beaumont plot on *P. avium* (mazzard cherry), *P. cerasifera* (myrobalan plum), *P. virginiana* var. *demissa, P. domestica* (except on French prune, which showed a few uredia on the old leaves late in December), *P. bokhariensis, P. hortulana, P. salicina,* and *P. marianna* (hybrid).

Fig. 3.—Cheesecloth chambers enclosing young trees of *Prunus* spp. inoculated with rust from various species of *Prunus.* Above the chambers are shown the containers from which water was conducted by means of cheesecloth wicks to the walls of the chambers, to provide moisture during the period of fungus incubation.

METHODS AND RESULTS OF ARTIFICIAL INOCULATIONS OF PRUNUS SPECIES

Thomas, Gilmer, and Scott (*10*) made artificial cross-inoculations with urediospores of *Tranzschelia pruni-spinosae* (probably of the discolor type) on detached leaves of different species of *Prunus.* Their results suggested that different strains of rust existed on *Prunus.* In order to test their results more conclusively, further experiments in the present series were conducted with growing plants under controlled conditions.

Small nursery trees of different species of ***Prunus*** (Lovell peach, Standard prune, Nonpareil almond, and others) were planted in peat and placed in cold storage from time of digging until the following September 1 (approximately 5 months), when they were transferred to 5-gallon containers and grown in a lathhouse. The trees, after transplanting, were surrounded at once with cheesecloth chambers (fig. 3) to protect them from outside sources of infection and to provide chambers that could be moistened by means of water conducted from overhead containers through cheesecloth wicks (fig. 3). Inoculation of these trees was made by first wetting the foliage with water. Twigs with rust-covered leaves were then moved about among the wet foliage and left suspended in the cheesecloth chambers. Dunegan and Smith (*5*) have determined that three hours are sufficient to give good germination of the urediospores from peach. The walls of the chambers were therefore kept moist for several hours after inoculation. The results of two years' experiments are presented in table 2.

TABLE 2

RESULTS OF ARTIFICIAL INOCULATIONS OF TREES OF *Prunus* SPP. IN LATHHOUSE CHAMBERS, WITH RUST (*Tranzschelia pruni-spinosae*) FROM VARIOUS SOURCES*

Host	Source of inoculum and results†					
	P. amygdalus (almond)	*P. fenzliana* (Fenzyl almond)	*P. persica* (peach)	*P. pumila* (sand cherry)	*P. insititia* (Damson plum)	*P. mahaleb* (mahaleb cherry)
Prunus alleghaniensis			+			..
P. amygdalus	+	+	+	+	+	.
P. angustifolia var. *Watsonii*	+	+	+	.	+	..
P. armeniaca	+	..	+	+	+	.
P. besseyi	..	.	+	..		.
P. bokhariensis	..	...	None	.	None	..
P. caroliniana	.	..	+	+		.
P. davidiana	..	..	+	.		.
P. domestica	Slight	+	Flecks	+	+	..
P. emarginata	+	..	..	..		..
P. fasciculata	..	..	..	.	+	..
P. fremontii	..	..	+	+	+	.
P. hortulana	..	..	None	..	None	.
P. mahaleb	+	..	+	+	.	..
P. maritima	+	+	+	+	+	..
P. mexicana	..	..	..	+	None	.
P. mume	..	..	..	..	+	..
P. munsoniana	+	..	+	.	+	..
P. orthosepala	..	..	..	+		..
P. persica	+	+	+	+	+	+
P. pumila	..	..	+	..		+
P. salicina	None	+	None	+	+	..
P. texana	..	..	+	+	+	..
P. umbellata	..	..	None	None	+	..

* Almond rust from trees of *P. amygdalus* at the Moreno plot and *P. fenzliana* at Riverside; peach rust from *P. persica* in Orange County, at San Jose, near Riverside, and at Hemet; plum rust from *P. insititia*, Citrus Experiment Station.

† + = positive results indicated by leaf spot, uredia, and urediospores.

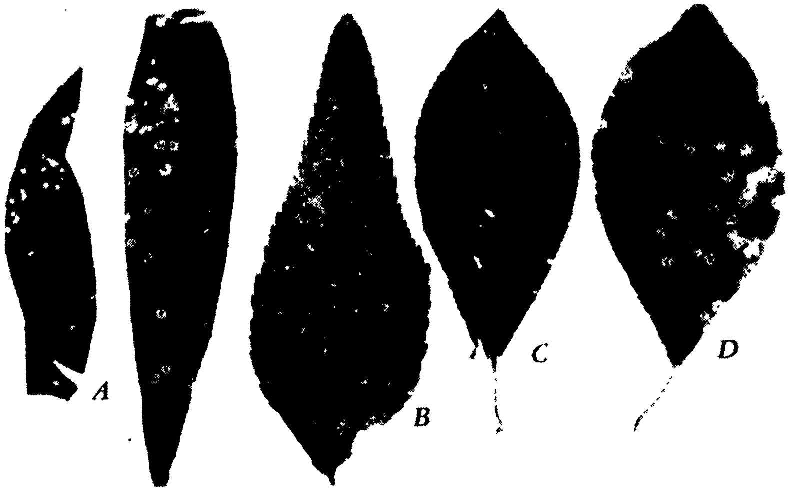

Fig. 4.—Rust infection on leaves of *Prunus* spp. artificially inoculated with rust from French prune: *A*, peach (*P. persica*); *B*, Nonpareil almond (*P. amygdalus*); *C*, Satsuma plum (*P. salicina*); and *D*, Standard prune (*P. domestica*).

TABLE 3

RUST INFECTION ON TREES OF *Prunus* SPP. THREE WEEKS AFTER INOCULATION MADE BY HANGING TWIGS WITH RUST-INFECTED LEAVES AMONG THE HEALTHY FOLIAGE DURING A NOVEMBER SHOWER

Host	Source of inoculum*	Infection†
Prunus amygdalus	Damson plum	+
P. amygdalus	Peach	+
P. angustifolia var. *Watsonii*	Damson plum	+
P. armeniaca	Damson plum	+
P. besseyi	Peach	+
P. bokhariensis	Peach	None
P. davidiana	Peach	+
P. domestica	Peach	None
P. domestica	Damson plum	+
P. fasciculata	Damson plum	+
P. fremontii	Damson plum	+
P. hortulana	Peach	None
P. mexicana	Peach	None
P. mume	Damson plum	+
P. salicina	Damson plum	+
P. texana	Damson plum	+

* *Prunus insititia* (Damson plum) and *P. persica* (peach).
† + = positive results indicated by leaf spot, uredia, and urediospores.

In another experiment, twigs with rust-covered leaves were tied among the healthy foliage of different species of *Prunus* during a November rain of several hours' duration. The results are presented in table 3.

The first indication of infection is the appearance of small yellow spots on the leaves about 25 days after inoculation. Artificial inoculations with rust

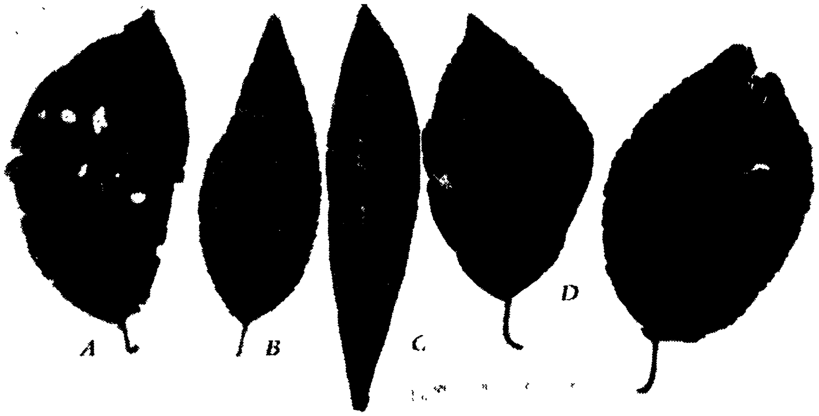

Fig. 5.—Rust infection on leaves of *Prunus* spp. artificially inoculated with rust from Damson plum (*P. insititia*): *A*, Satsuma plum (*P. salicina*, limited infection); *B*, almond (*P. amygdalus*); *C*, peach (*P. persica*); and *D*, Standard prune (*P. domestica*).

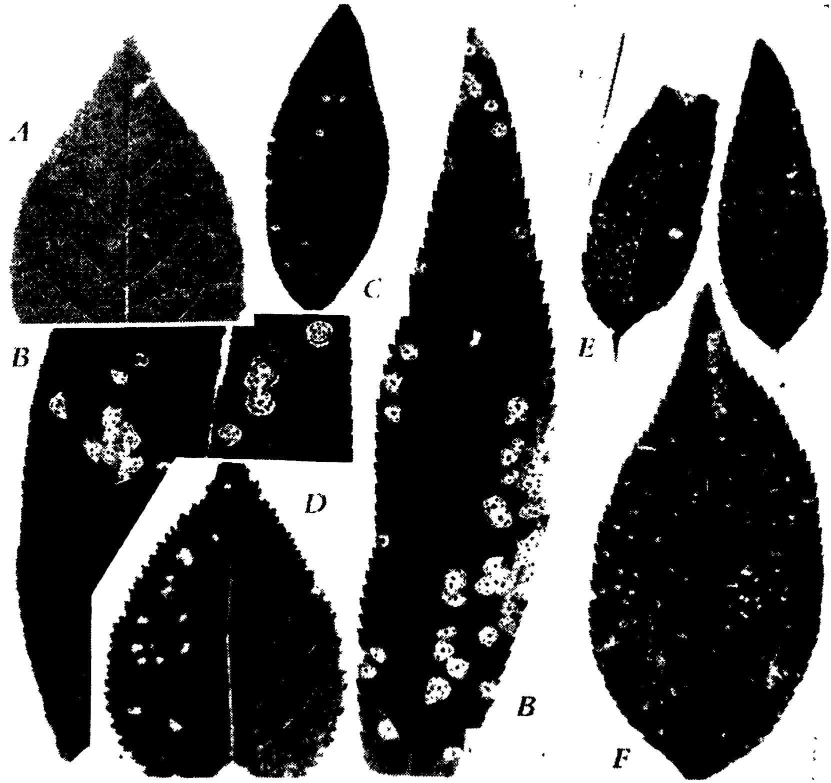

Fig. 6.—Rust infection on leaves of *Prunus* spp. artificially inoculated with rust from peach: *A, P. maritima* (lesions poorly developed); *B, P. persica* (note circular arrangement of the uredia); *C, P. angustifolia; D, P. armeniaca; E, P. amygdalus* (bitter almond); *F, P. alleghaniensis.* Inoculations of *E* and *F* were with rust from Brownwood, Texas.

from peach, almond, prune, and Damson plum caused typical rust spots on leaves of Lovell peach. Positive results of rust infection on leaves of peach, almond, Satsuma plum, and Standard prune with spores from French prune and Damson plum (figs. 4 and 5) have been produced repeatedly and have thus differed from the results of Thomas, Gilmer, and Scott (*10*), who report negative results on almond, peach, and cherry with spores from prune.

As shown in figure 6 and table 2, rust from peach is pathogenic in different degrees on different species of *Prunus*. On almond, this rust gave yellow leaf spots and a fair degree of infection, with the sori usually somewhat better developed than those of almond rust on peach. Rust from peach, from Hemet, with telia and uredia, produced positive results on almond, apricot, and peach (table 4). Results of peach rust on Standard prune were so slight, however, that they might be considered to be negative: some very small spots could be detected with a hand lens, and a few uredia were found near the leaf margins.

TABLE 4

RESULTS OF ARTIFICIAL INOCULATION OF ALMOND (*Prunus amygdalus*), APRICOT (*P. armeniaca*), AND PEACH (*P. persica*) WITH RUST SPORES FROM SOURCES INDICATED

Source of inoculum	Positive results of inoculations on		
	Almond	Apricot	Peach
Peach (Moreno)	Abortive sori	Yellow spots, 3–5 mm diam.	Yellow spots, 3–10 mm diam.
Peach (Hemet)*	Abortive sori	Yellow spots, 3–5 mm diam.	Yellow spots, 3–5 mm diam.
Peach (Hemet)*	Abortive sori	Yellow spots; fruiting	Yellow spots, 1–2 mm diam.
Almond (Moreno)	Sori plump and numerous	Sori poorly developed	Yellow spots, 1 mm or less in diam.; fruiting scant
French prune (San Jose)	Slight infection; fruiting	Yellow spots	Yellow spots, 1 mm or less in diam.
French prune (San Jose)	Sori; fair fruiting	Well-developed yellow spots	Yellow spots; sori plump; fruiting
Sugar plum (San Jose)	Sori few but well formed	Slight fruiting	Yellow spots
Damson plum (Riverside)	Spots; slight fruiting	Purple spots	Purplish spots

* Teliospores numerous.

Inoculations with rust from almond (fig. 7) were positive on peach (*Prunus persica*), almond (*P. amygdalus*), and apricot (*P. armeniaca*), and on some other species of *Prunus* (tables 2 and 4). On peach leaves the rust spots resembled rust from peach on peach. The uredia were often poorly developed, but were sometimes of normal size. Telia were observed in limited numbers on almond, but not on peach or apricot. Small spots were apparently developed on some of the leaves of Standard prune, but these were less than 1 mm in diameter, were without uredia, and their cause could not be determined with certainty. In fact, the rust was so poorly developed on Standard prune as best to be regarded as negative. Rust from almond gave negative results on *P. salicina*. Results with rust from *P. fenzliana* (table 2) were similar to those with rust from almond, except for positive results (small spots) on *P. salicina.*

Rust inoculations with spores from *Prunus domestica* (French prune) and from *P. insititia* (Damson plum) gave similar results (figs. 4 and 5) on the different hosts tested. The two rusts have a similar appearance on the leaves of their respective hosts and develop telia late in the season. Rust inoculations from French prune produced small spots 1 to 3 mm in diameter on leaves of Nonpareil almond (fig. 4, *B*). A few uredia and spores were produced, but the sori were often white and undeveloped, no telia were observed, and the rust development was abnormal. This same rust formed small spots, with uredia and spores, on leaves of Satsuma plum (fig. 4, *C*). On peach leaves (figs. 4, *A*, and 5, *C*) rust from French prune and from Damson plum readily produced spots and spores characteristic of rust from peach on peach.

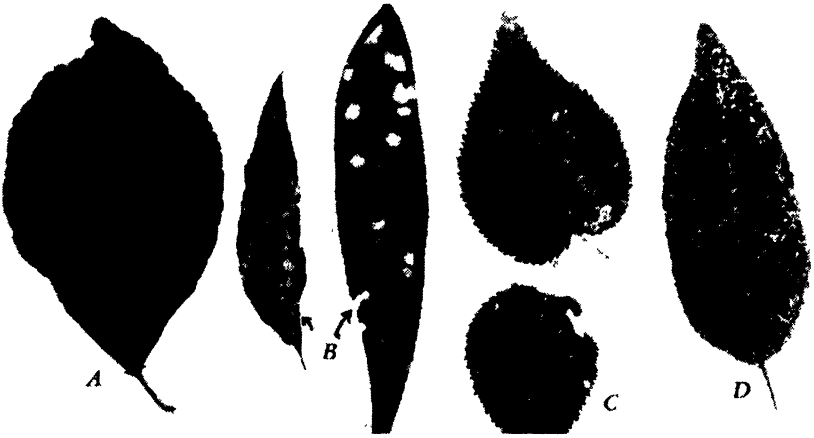

Fig. 7.—Rust infection on leaves of *Prunus* spp. artificially inoculated with rust from almond: *A,* Standard prune; *B,* peach; *C,* apricot; *D,* almond.

The very light infection resulting when Standard prune (*Prunus domestica*) was inoculated with rust from almond and peach (*P. persica*), and the severe infection when Standard prune was inoculated with rust from plum, suggest that these rusts may represent two different strains.

VARIABILITY OF UREDIA

Observations in the present experiments indicate that the uredia of the plant rust *Tranzschelia pruni-spinosae* from different sources are variable in size and color when growing on different species of *Prunus*. Similar differences in size have also been observed by Cristinzio (*3*) in Italy.

Natural rust infection on Wickson plum (of unknown source) had lesions 1 to 2 mm in diameter, with white uredia and few urediospores. Rust from Damson plum developed white, abortive uredia on almond and on Satsuma plum, where often only small flecks were formed. It is probable that there is considerable variation in the size and color of the uredia on other Japanese plums. The uredia on the semiwild species of *Prunus persica* (P.I. 40001 and 40864) and on J. H. Hale peach were small in comparison with those on Lovell peach seedlings. The uredia on Lovell peach were larger (0.5 to 3. mm diam.) and more numerous than the sori on J. H. Hale (0.5 to 1 mm diam.).

The commercial almonds of California also show variation in the uredia. The variety having the smallest, poorest-developed uredia is Ne Plus Ultra; the I.X.L. variety has medium-sized uredia, while uredia on Texas and Nonpareil are large and well developed. *Prunus fenzliana* (P.I. 27336) has large, well-developed uredia. On peach leaves, with peach rust (fig. 6, *B*), the uredia often have a circular arrangement on the leaf spot.

TABLE 5

SPECIES OF *Prunus* ON WHICH THE RUST *Tranzschelia pruni-spinosae* (DISCOLOR TYPE) HAS BEEN OBSERVED IN CALIFORNIA*

Species	Description	Species	Description
Prunus amygdalus	Nonpareil almond*	*P. mira*	Seedling*
P. amygdalus	P.I. 29218*	*P. mume*	P.I. 47950*
P. amygdalus	P.I. 30408	*P. mume*	P.I. 46694*
P. andersonii	Seedling	*P. munsoniana*	Seedling
P. angustifolia†	Seedling	*P. persica*	P.I. 40001*
P. armeniaca	Royal apricot	*P. persica*	P.I. 40000
P. armeniaca	P.I. 39464	*P. persica*	P.I. 41395
P. armeniaca var. *mandshurica*	Manchurian apricot	*P. persica*	P.I. 34685
P. besseyi‡	Western sand cherry	*P. persica*	Lovell peach
P. brigantina	P.I. 65051	*P. persica* x *P. amygdalus*	Hybrid almond x Peento peach
P. bucharica	P.I. 30975	*P. persica*	Flat peach
P. cerasifera	Myrobalan plum*	*P. persica*	Indian peach*
P. dasycarpa‡	Purple apricot	*P. persica*	Peento hybrid*
P. davidiana	P.I. 36664*	*P. pumila*	Seedling*
P. davidiana	P.I. 26604	*P. salicina*	Wickson plum
P. domestica	Clyman plum	*P. salicina*	Santa Rosa plum
P. domestica	French plum*	*P. salicina*	Satsuma plum
P. domestica	Reine Claude*	*P. sibirica*‡	P.I. 68819
P. domestica	Tragedy plum*	*P. simonii*	P.I. ?
P. emarginata	Seedling*	*Prunus* sp.	Marianna*
P. fasciculata	Seedling*	*Prunus* sp.	P.I. 55941
P. fenzliana	P.I. 27336*	*Prunus* sp.	P.I. 40864*
P. fremontii	Seedling*	*P. subcordata*	Seedling
P. insititia‡	Black Bullace*	*P. tangutica*	P.I. 40010
P. kansuensis	P.I. 68976*	*P. texana*‡	Seedling*
P. mahaleb‡	P.I. Lucie*	*P. umbellata*	P.I. 38974
P. mexicana	Seedling		

* The asterisk indicates that the discolor type of teliospore was observed.
† Infected by artificial inoculation.
‡ Specimen collected at San Jose, California. Unless otherwise specified, all material was collected in southern California, mostly at Riverside and Whittier.

OBSERVATIONS OF RUST ON DIFFERENT SPECIES OF PRUNUS IN CALIFORNIA

The different species of *Prunus* on which rust has been observed in California are listed in table 5. The discolor type was the only one found, and probably is the only form occurring in California. Herbarium specimens have been deposited in the collection at the University of California Citrus Experiment Station at Riverside, and duplicate specimens have been deposited in the herbarium at the University of California at Berkeley.

Smith and Cochran (*9*) have shown that the native California species *Prunus andersonii, P. emarginata, P. fasciculata, P. fremontii,* and *P. subcordata* are susceptible to the discolor type of rust when grown under cultivation and subject to natural infection. *P. virginiana* var. *demissa* is apparently not attacked. Smith (*8*) was successful in causing rust spots on the leaves of *P. ilicifolia, P. lyonii,* and *P. caroliniana.* There is no record of infection of any of these species in their natural habitats.

Certain species of *Prunus* native to the eastern United States were but slightly attacked with rust when grown in California under cultivation; others were extremely susceptible. Infected leaves were rare on *P. munsoniana, P. umbellata,* and *P. angustifolia* var. *Watsonii.* Artificial inoculation of the variety *Watsonii* (table 2), however, indicated that it was somewhat susceptible. *P. pumila, P. besseyi,* and *P. maritima* were extremely susceptible.

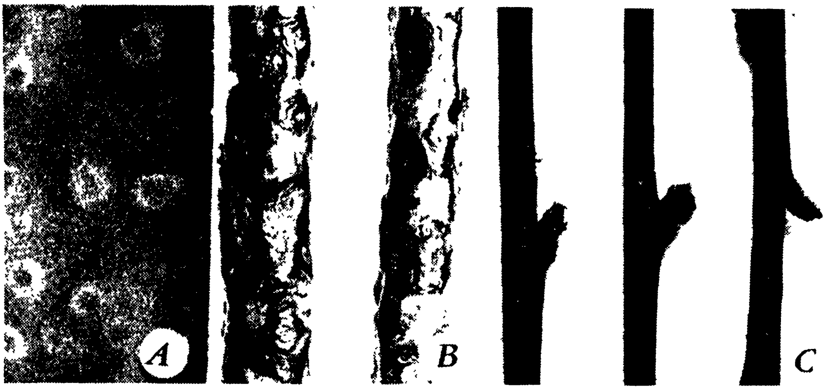

Fig. 8.—Natural rust infection on (*A*) fruit and (*C*) twigs of *Prunus persica,* and on (*B*) stems of *P. mume.* (*A* and *C* photographed by J. T. Barrett.)

Leaves of *P. mexicana* had brownish, circular spots, 1 to 3 mm in diameter, on which uredia developed; and urediospores from these spots were discernible under the microscope. Seedlings of *P. orthosepala* were almost free from rust infection. Some of the leaves on a few of the seedlings had small brownish spots, 1 to 2 mm in diameter, that showed poorly developed uredia.

The rust on peach (*Prunus persica*) usually shows only uredia. Telia, when formed, are seldom abundant; they were plentiful at times, however, on peach growing near Hemet. On two semiwild species from China (P.I. 40001 and 40864), the rust caused small purple spots, 1 to 2 mm in diameter, that had a few (usually 1 to 3) uredia. Only rarely were the leaves of these plants covered with rust as early or as completely as were those of the Lovell seedlings. The peach fruit (fig. 8, *A*) is occasionally attacked by rust, which causes circular spots with lighter-colored margins. Peach twigs (fig. 8, *C*) are also attacked, and the mycelium carried on the twigs through the dormant season serves as an initial source of inoculum for the new plant growth.

Seedlings of *Prunus fremontii* growing near *P. cerasifera* var. *Pissardii* produced trees having leaf characters closely resembling those of *P. cerasifera* and *P. cerasifera* var. *Pissardii* and were probably first-generation hybrids between these species and *P. fremontii.* All these seedlings were extremely susceptible to rust (of the discolor type, where teliospores were present and the type could be determined).

Rust on *Prunus domestica* develops uredia, followed by telia in abundance and almost a complete absence of uredia. Rust on almond (*P. amygdalus*), like that on *P. domestica,* produces uredia and later abundant telia.

Prunus caroliniana, an evergreen species native to the eastern United States, is susceptible to rust in California. Smith (*8*) has shown that *P. caroliniana* is naturally infected when grown near other species of *Prunus* that carry the inoculum. It has also been successfully infected artificially. The rust fruits sparingly, however, and the diseased tissue falls out and leaves a shot-hole effect.

Rust has been severe on *Prunus fenzliana* at the Citrus Experiment Station, and has caused early infection and marked defoliation. Leaves of the peach rootstock had definite spots with uredia, but defoliation was slight. The rust on *P. fenzliana* was apparently that of almond and it showed the development of telia, a characteristic of the rust as it develops on almond.

Prunus ilicifolia, a California evergreen species, has been successfully infected artificially by rust, according to Smith (*8*). Fruiting was sparse, but brownish spots surrounded with yellow-greenish zones were readily produced on the young leaves, though not on the older leaves.

Rust and definite lesions developed on the stems (fig. 8, *B*) of some of the seedlings of *Prunus mume* grown at the Citrus Experiment Station, but rust does not now appear to be present on the stems of the mature trees. Rust has been observed on leaves of other trees of *P. mume* at the Citrus Experiment Station.

Rust has not been observed occurring naturally in California on the following species: *Prunus bokhariensis* (P.I., 40223, 40224, and 40231), *P. avium* (successfully infected artificially with rust from *P. pumila*), *P. cerasus, P. hortulana, P. serotina, P. virginiana* var. *demissa, P. ilicifolia, P. yedoensis, P. majestica* Koehne (P.I. 55417), and *P. mira* (P.I. 34601). Tranzschel and Litvinov (*11*) state that forms of *Tranzschelia* are generally unknown on the cherry in Europe.

Other species of *Prunus* may sometimes show a shot-hole effect on the leaves as the result of an attack by rust. Evidence of this is seen in partially excised tissue on which rust sori are present. This effect has been noted on the two semiwild species of peach from China (P.I. 40001 and 40864) and on almond and apricot trees that were inoculated with urediospores from prune (*P. domestica*) and Damson plum (*P. insititia*).

SUMMARY

There are two different types of the rust *Tranzschelia pruni-spinosae* on *Prunus* spp., namely, the typica and the discolor types, as described by Dunegan (*4*). The discolor type is the only one that has been observed in California.

In the studies reported in the present paper, artificial inoculations of *Prunus* spp. with rusts from almond, peach, prune, and Damson plum usually showed some pathogenicity, although the development of the spots, the uredia, and the amount of fruiting were often far different on the different cultivated hosts. When species of *Prunus* were inoculated, the uredia were at times abortive. Peach and almond were susceptible to rust from almond, peach, and

prune. Standard prune and other varieties of *P. domestica,* as well as Damson plum (*P. insititia*), were susceptible to rust from prune and from Damson plum. Rust from peach and almond gave only light infection on Standard prune, in the form of very small spots that were so inconspicuous as to be readily overlooked, and that did not develop sori, except possibly a few near the leaf margin, but peach is severely infected by rust from Standard prune and Damson plum. Rust symptoms on almond are very similar to those on peach, the chief dfference being in the abundant production of telia sori on almond.

Orchard observations and results from interspecific host inoculation tests indicate that the rusts on peach (*Prunus persica*) and on Standard prune (*P. domestica*) may represent at least two different strains.

ACKNOWLEDGMENTS

The writer wishes to express appreciation to Dr. L. C. Cochran, who provided experimental trees and gave many helpful suggestions during the investigation, and in whose experimental plots of *Prunus* spp. many of the observations on rust were made. Thanks are extended also to Dr. B. A. Rudolph, and to Mr. L. R. Cody, County Agricultural Commissioner, San Jose, for rust material on *P. domestica.* The writer is especially obligated to Dr. John C. Dunegan, who supplied a copy of the paper by Tranzschel and Litvinov (*11*), translated into English, from the Russian, by Dr. S. L. Jodidi (mycological terminology revised by John C. Dunegan).

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SOD WEBWORMS AND OTHER LAWN PESTS IN CALIFORNIA[1]

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INTRODUCTION

AFTER the summer of 1929, various publications began to record widespread damage to lawns and golf courses by certain species of the pyralid genus *Crambus*. In 1931, outbreaks of unprecedented magnitude occurred in many states, and the sod webworms, more popularly known as lawn moths, became recognized as major lawn pests.

In California, sod webworms have been reported as causing more or less severe injury every year since 1929. The damage to private lawns, parks, and golf greens cannot well be evaluated in money. Undoubtedly, hundreds of thousands of dollars have been expended in this state in controlling this pest and replacing the injured grass.

Lacking accurate information on lawn insects in California, pest-control operators have failed to agree on control practices. The author, accordingly, studied the subject from the fall of 1938 through 1941 in order to summarize the existing knowledge of such pests, present new biological data, and suggest practical means of control.

ECONOMIC HISTORY OF SOD WEBWORMS

The genus *Crambus* is practically world-wide in distribution and many of its species are recognized as agricultural pests. *Crambus* species have injured lawns and rice in Mauritius, pasture in Norway and England, sugar cane in Queensland, and rice in Brazil. In North America about 100 species are known, and some had attacked corn, oats, wheat, and pasture land as early as 1850. By 1895 the cranberry girdler, *C. hortuellus* (Hbn.) was a problem along the Atlantic seaboard. A few years later the corn root webworm, *C. caliginosellus* Clem. became a prominent pest of tobacco. Although Felt (1894)[3] pointed out that the members of the genus *Crambus* are predominantly grass feeders, their injury to pasture received only an occasional comment till the publication of G. G. Ainslee's studies beginning in 1917.

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[1] Received for publication March 17, 1943.

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[3] See "Literature Cited" for complete data on citations mentioned in the text by author and date of publication.

Damage by webworms to lawns or golf courses was recorded only twice, apparently, before 1929. Moznette (1921) reported injury to Florida turf by *Crambus haytiellus* Zinck. Ainslee (1922*a*) mentioned sod webworms as occasional pests of lawn grass, "especially in times of deficient rainfall." In the summer of 1929, however, and in the following four summers, damage to lawn grass by species of *Crambus* was widespread in North America. Outbreaks during these years apparently coincided with the drought that prevailed over much of the country. Noble (1932) suggested that the injury to lawns and golf greens from the excessive numbers of sod webworms in 1931 "was due to their gradual concentration in artificially watered areas of grass during the dry summers of 1930 and 1931, and to the unusually large numbers of the overwintering forms which survived the mild weather of the preceding winters."

The first published report of sod-webworm damage to California lawns (Brock, 1929) also coincided with the beginning of a series of relatively dry years. Between 1928 and 1934, although California did not participate so fully in the general drought as some other sections, there was much less rain here than usual. According to U.S. Weather Bureau reports the average annual rainfall deficit from July, 1928, to June, 1934, was 32.1 per cent in Sacramento, 22.5 per cent in San Francisco, 26.0 per cent in Fresno, and 9.0 per cent in Los Angeles. Since lawns and golf courses are always irrigated, the moths may congregate in these areas in dry years. Brock (1929) reported that during the summer of 1929 bent-grass and bluegrass lawns were injured in Orange County. Both *Crambus bonifatellus* and *C. sperryellus* (misidentified as *C. cypeidalis*) were present. Keifer (1931) noted *C. sperryellus* (misidentified as *C. leachellus*) as damaging lawns in San Joaquin County "during the latter part of summer and early fall" of 1931. Although published records are not available for 1931 to 1938 in California, sod webworms took a yearly toll of lawns and golf courses in many sections, according to greenkeepers and pest-control operators interviewed during the present study. In the summer of 1939, extensive injury in southern California was observed by the writer. Unofficial estimates by county authorities handling complaints from homeowners placed the damage in Los Angeles County alone at more than $75,000. After the heavy rainfalls of 1939–40 and 1940–41, infestations were less severe. Although injury during the summer of 1941 was reported throughout the Sacramento and San Joaquin valleys and in the coastal district from Santa Rosa south to San Diego, outbreaks were apparently only local.

GEOGRAPHICAL DISTRIBUTION

Crambus bonifatellus.—Hulst (1887) originally described *Crambus bonifatellus* from Colorado. Dyar (1904) recorded it from Ainsworth, Bear Lake, and Revelstoke in British Columbia. According to A. B. Klots,[4] an authority on *Crambus, C. bonifatellus* appears to occur throughout the Canadian Zone of the entire Rocky Mountain and Great Basin area. Out-of-state specimens in the writer's collection are from Hubbard, Forest Grove, and Corvallis, Oregon. Keifer (1931) first recorded this species in California. The known distribution here is indicated in figure 1, *B*. In general it occurs in a narrow coastal belt no more than 50 miles wide. Exceptional localities are Sacramento, Oroville, Goodyear Bar, and Lake Tahoe. The Sacramento and Oroville specimens were collected by H. H. Keifer, who told the writer that the species was extremely rare in these localities. All inland records are recent (1926 to 1941), and the specimens have been associated with lawns. Collections along the coast were noted as early as 1908 (San Francisco) and 1913 (San Diego, W. S. Wright), but without host designation.

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[4] Personal correspondence.

Crambus sperryellus.—*Crambus sperryellus* is apparently restricted mainly to California. Here, however, it is more widely distributed than *C. bonifatellus*.

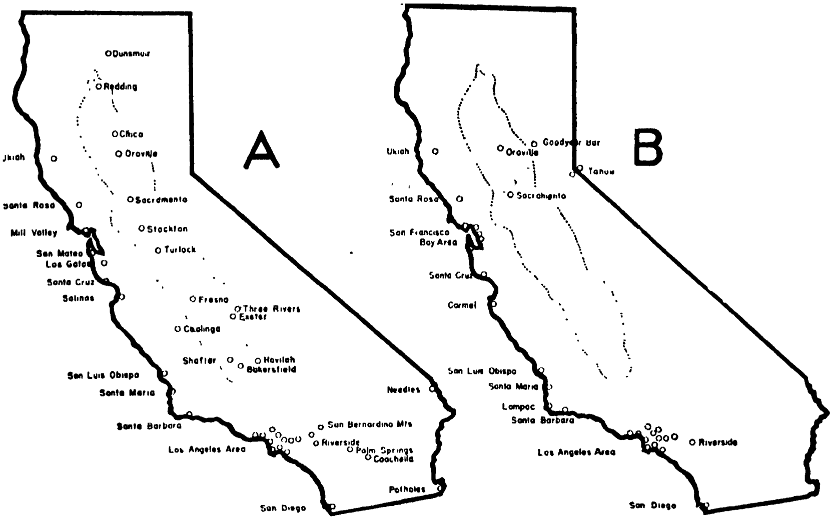

Fig. 1.—Distribution map of California lawn moths: *A*, *Crambus sperryellus*; *B*, *Crambus bonifatellus*. The dotted lines indicate the approximate boundary of the Sacramento and San Joaquin valleys.

Klots (1940) records it from Dunsmuir, Sacramento, Santa Cruz, Three Rivers, Exeter, Havilah, Saugus, the Los Angeles area, Claremont, the San Bernardino Mountains (6,000 feet), Colton, Riverside, Palm Springs, Coachella, Needles, and San Diego, as well as Tempe, Arizona. The writer has seen specimens from Grants Pass, Oregon, and from the additional California localities marked on the map, figure 1, *A*. The species abounds in the Sacramento and San Joaquin valleys as well as near the ocean. On the coast and in southern California as far inland as Riverside, both species may fly together; but, as a rule, one or the other will predominate on a given lawn or golf course.

HOST-RANGE STUDIES

Lawns in California are usually of Bermuda grass, orchard grass, Kentucky bluegrass, Australian ryegrass (*Lolium* spp.), or creeping bent. Bent and bluegrass are generally conceded to make the finest turf. Australian ryegrass is relatively inexpensive, but individual plants tend to segregate, so that the ground cover may not be smooth. In some districts ryegrass grows so poorly, especially from cheap seed, that the sparse appearance is frequently mistaken for lawn-moth damage. Bermuda grass and orchard grass ordinarily appear first as weeds, but once established will make serviceable lawns.

Field Observations on Host Preferences.—In the writer's experience only bent-grass and young bluegrass lawns have been injured by sod webworms. Although young Bermuda grass lawns have occasionally been reported as attacked, he has seen no instances personally. An attempt was made to determine whether the apparent immunity of Bermuda grass, orchard grass, and ryegrass resulted from a paucity of sod-webworm larvae or from a greater resistance of the grass. Whenever control experiments were conducted on bent or bluegrass, adjacent lawns were searched for the larvae with a pyrethrum testing solution.[5] Apparently Bermuda grass and Australian ryegrass lawns seldom harbored more than 2 or 3 *Crambus* larvae to the square yard even when they adjoined severely infested bent or bluegrass lawns. On the other hand, orchard grass sometimes had a high population without showing injury. Though the leaves are tender, the individual plants are very large as compared with bluegrass, and size may account for the greater resistance. Bluegrass lawns a year old or older were ordinarily not injured perceptibly even in the worst-infested districts. As a rule, such plots contained only about 25 larvae

TABLE 1

LABORATORY HOST-RANGE STUDIES ON *Crambus bonifatellus* AND *Crambus sperryellus**

Host plant	*Crambus bonifatellus*		*Crambus sperryellus*	
	Instar attained	Average time from eclosion to pupation	Instar attained	Average time from eclosion to pupation
		days		*days*
White clover (*Trifolium repens*)	6	23.0	8	39.5
Orchard grass (*Dactylis glomerata*)	7	26.0	7†	....
Creeping bent (*Agrostis palustris*)	6	27.0	6†	....
Wheat (*Triticum sativum*)	6	27.0	8†	....
Kentucky bluegrass (*Poa pratensis*)	6	29.0	8	34.5
Australian ryegrass (*Lolium* spp.)	7	31.5	7	34.0
Bermuda grass (*Cynodon dactylon*)	7	34.0	7	31.0
Crabgrass (*Digitaria sanguinalis*)	8	37.0	9†	....
Rescue grass (*Bromus catharticus*)	7†	....	8	40.5
Qat (*Avena sativa*)	7†	..	7†	...
Corn (*Zea mays*)	5†	....	7	42.0
Ripgut grass (*Bromus rigidus*)	1†	....	6†	....
Pampas grass (*Cortaderia selloana*)	1†	...	1†	....
Barley (*Hordeum vulgare*)	1†	...	1†	....
St. Augustine grass (*Stenotaphrum secundatum*)	1†	....	1†	....
Barnyard grass (*Echinochloa crus-galli*)	1†	...	7	34.0
Dichondra (*Dichondra repens*)	1†	..	1†	....

* In each test 15 newly hatched larvae were used. Room temperatures of 73° to 77° F prevailed through the experiment.

† All specimens on this plant species died before pupation.

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[5] The testing solution was prepared from a commercial water-miscible extract containing about 2 per cent pyrethrins, diluted 1 part to 400 parts of water (equivalent to about 2 teaspoons of extract to a gallon of water). The mixture was applied to grass with a sprinkling can, 1 quart to ¼ square yard.

or fewer to the square yard, though in one case an average of 104 were causing no apparent damage. The resistance of old lawns can be attributed to the better establishment of the plants and to a greater toughness, which makes the bluegrass less suitable as larval food.

Laboratory Studies on Host Range.—In the search for more definite information on the host range of the two California species of lawn-infesting *Crambus,* specimens were reared in the laboratory on cut leaves of various grasses and other plants—sweet corn, orchard grass, ripgut grass, rescue grass, Kentucky bluegrass, pampas grass, Australian ryegrass, wheat, barley, Bermuda grass, oat, creeping bent, St. Augustine grass, crabgrass, barnyard grass, white clover, and *Dichondra repens.* Table 1 presents the results.

TABLE 2

LABORATORY TESTS ON LARVAL FOOD PREFERENCES

Host plant	Number of larvae feeding on a given host*					
	Crambus bonifatellus			*Crambus sperryellus*		
	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
	larvae	*larvae*	*larvae*	*larvae*	*larvae*	*larvae*
Kentucky bluegrass	16	25	..	14	21	..
White clover	10	..	22	6	.	9
Orchard grass	4	.		10		.
Australian ryegrass	.	4	6	..	6	18
Bermuda grass	.	1	2	..	3	3

* Thirty larvae were used in each test.

Neither species of moth could be reared beyond the first instar on pampas grass, barley, St. Augustine grass, or *Dichondra repens,* nor could *Crambus bonifatellus* live on barnyard grass or ripgut grass. *C. sperryellus* was apparently better able to use coarser food such as ripgut grass, Bermuda grass, and barnyard grass. The inability of *C. sperryellus* to complete development on creeping bent does not agree, however, with its behavior in lawns, where this grass is the type most susceptible to infestation.

These experiments, in which larvae were offered only one type of food, present a clearer picture of host potentialities than of food preferences. Further tests, accordingly, were made to determine the result of offering larvae a choice among five common lawn plants (table 2). For *Crambus bonifatellus* the order of preference was Kentucky bluegrass, white clover, orchard grass, Australian ryegrass, and Bermuda grass; and for *C. sperryellus* the order was the same except that clover was fourth on the list. The relative distaste shown by both species for Australian ryegrass and Bermuda grass agreed with field observations, but the apparent fondness of *C. bonifatellus* for clover was surprising, since no injury to this plant has been reported.

DIAGNOSIS OF SOD-WEBWORM INFESTATION

Lawn grasses are susceptible to many entomological, pathological, and physiological ills. These troubles are often confused and incorrectly treated, with resultant loss of time and money. The usual restriction of lawn moths to bent-grass lawns or to bluegrass lawns not over a year old, somewhat simplifies the problem.

To identify lawn-moth damage, the writer used the following criteria: (1) Irregular brown patches were usually present instead of the circular spots associated with fungus. (2) On close examination the grass near the dead areas evidenced larval feeding by its shortness or uneven height. (3) Numerous adult moths were present, resting in the shrubbery or under window ledges near the lawn. (4) Predatory yellowjackets or parasitic wasps and flies, if abundant, pointed to a high population of host larvae. (5) Green areas near damaged sections of the lawn were tested with a pyrethrum solution (footnote 5, p. 270). This fifth criterion was by far the most reliable: if at least 100 sod-webworm larvae to the square yard wriggled to the surface within 10 minutes, a serious infestation was indicated.

Three rather unsatisfactory methods of diagnosis were sectioning, flooding, and the use of hot water. In the first, a section of sod was removed with a knife, and the grass was torn apart. The task was not easy, however, unless the larval population was extremely high; often the small specimen of sod that could feasibly be examined gave an erroneous idea of the infestation. In the second method a suspected section of lawn was flooded with a hose for 15 minutes or more to drive the larvae to the surface. This procedure, however, was inefficient. When very hot water was poured on, the larvae wriggled to the surface; but such treatment damaged the grass.

Two phenomena that were not reliable, being often associated with relatively healthy lawns, were the presence of adult lawn moths at dusk, and the visitations of blackbirds in flocks during the day.

TAXONOMY

Although *Crambus* moths and related genera are sometimes separated in a special family, Crambidae, recent workers treat them as the subfamily Crambinae of the family Pyralidae. The species of *Crambus* fold their wings in repose closely about the body, and have prominent porrect labial palpi. Of the eighteen species reported from California, only two, *C. bonifatellus* (Hulst) and *C. sperryellus* Klots, are known to infest lawns. Their systematic history is as follows:

Crambus bonifatellus (Hulst)

Spermatophthora (?) *bonifatella* Hulst, 1887, Ent. Amer. 3:135.
Crambus bonifatellus Fernald, 1896, Massachusetts Agr. Col. Spec. Bul. p. 57.

Apparently *Crambus bonifatellus* has no close relatives. The brownish forewing variegated with buff, whitish, and black distinguishes it from all other North American species (fig. 2, *A* and *B*).

Crambus sperryellus Klots

Crambus cypridalis of Brock, 1929. California State Dept. Agr. Monthly Bul. 18:574 (based on misidentification).

Crambus leachellus of Keifer, 1931. California Dept. Agr. Monthly Bul. 20:617 (based on misidentification).

Crambus sperryellus Klots, 1940. Southern California Acad. Sci. Bul. 39:62.

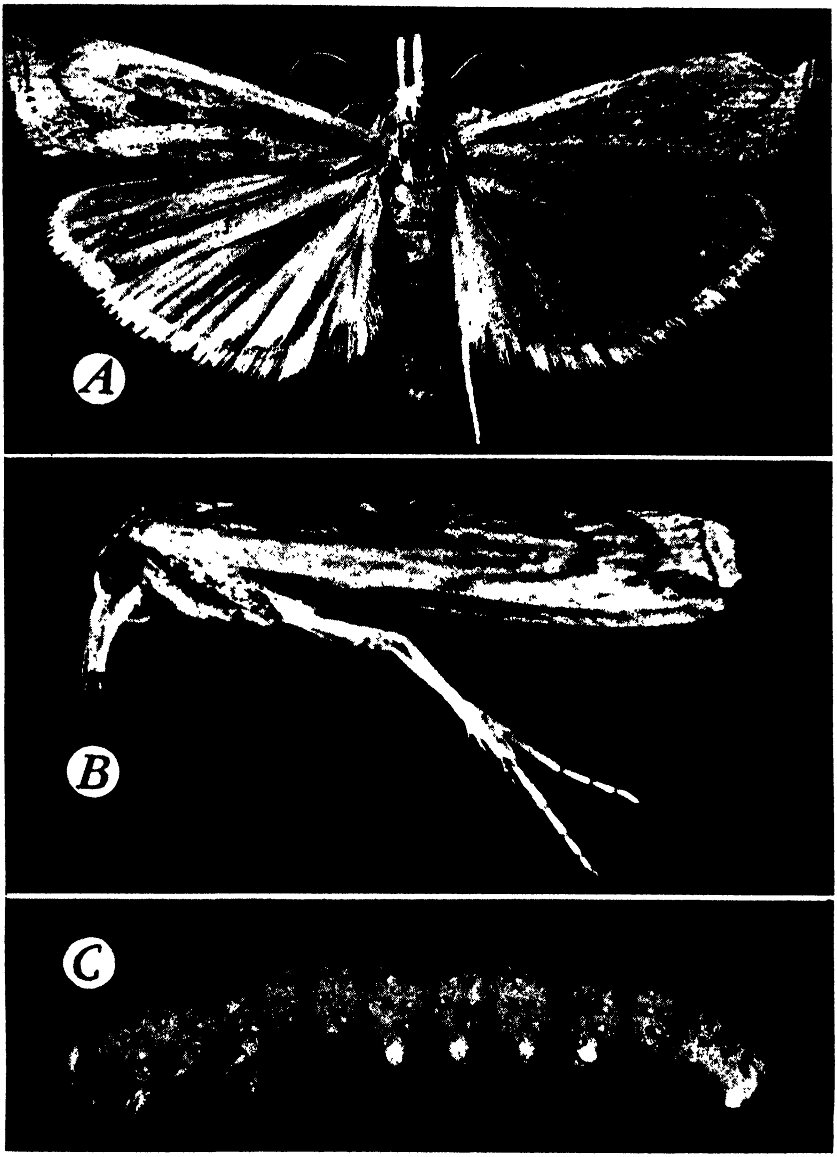

Fig. 2.—*Crambus bonifatellus* (Hulst): *A* and *B*, adult female; *C*, sixth-instar larva.

Crambus sperryellus belongs to a group of species having a longitudinal silver stripe on the forewing. *C. sperryellus* is closely related to *C. leachellus* Zincken and *C. occidentalis* Grote, which also occur in California. From these, however, it is distinguished by the presence in its forewing of a distinct whitish or yellowish-white shade connecting the proximal end of the forewing stripe with the outer wing margin (fig. 6, *A* and *B*).

Common Names.—Through newspapers, garden magazines, and insecticide salesmen the two California species of lawn-infesting *Crambus* have become well known as "lawn moths." No distinction has been made between them, however, even though the adults are readily identified by their color pattern. They will here be designated as the fawn-colored lawn moth (*C. bonifatellus*) and the silver-barred lawn moth (*C. sperryellus*).

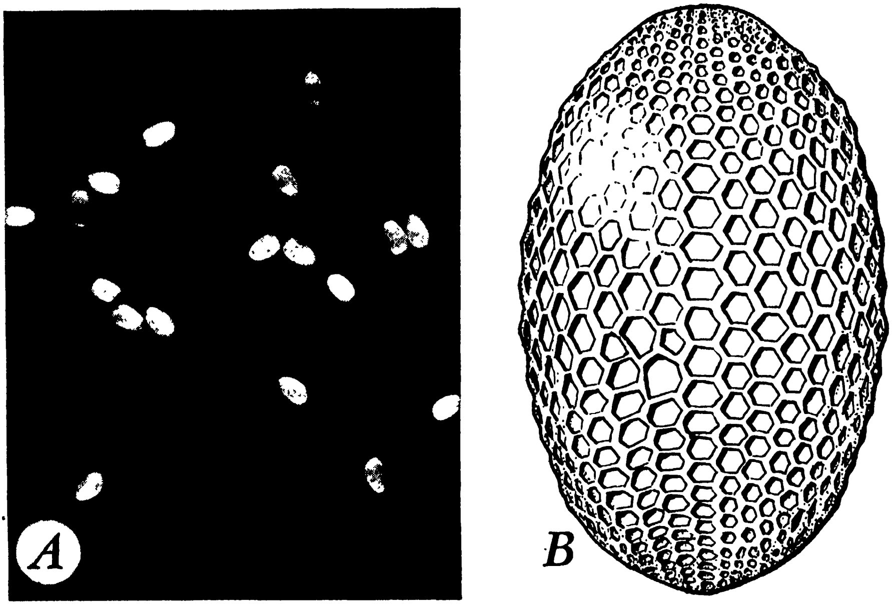

Fig. 3.—*A*, Eggs of *Crambus bonifatellus* (enlarged); *B*, drawing of egg, much enlarged to show the sculpture of the chorion.

TECHNICAL DESCRIPTION OF STAGES OF CRAMBUS BONIFATELLUS

Adult Female.—(Fig. 2, *A* and *B*) Ground color of forewing and body in general creamy buff, ground color of hind wing somewhat more silvery; forewing with three irregular and partially connected spots along basal half of cubitus, an irregular streak or spot posterior to and paralleling R_5, a triangular dark spot apically, a small oblique black spot between Cu_1 and Cu_2 subapically in the otherwise brown subterminal line, and eight small black spots of terminal line marking ends of veins at wing margin; three to five irregular spots of white scales in discal area of wing; fringe buff and brown. Hind wing with silvery buff ground color overlaid with scattered grayish-brown scales becoming thicker apically; fringe silvery gray. Wing expanse 20 to 23 mm.

Adult Male.—Markings as in female except that ground color of forewing and body in general is buff brown, a black streak along basal half of cubitus is less distinct, fringe of forewing is mostly brownish, hind wing has a darker cast and its fringe is darker. Antenna not pectinate, but twice as thick as that of female; wing expanse 17 to 20 mm. For the genitalia, see also figure 5, *D*, *E*, and *F*. Tegumen broadly convex. Uncus stout, 1½ times as long as gnathos; with a short sharp downward-pointing apex; a few short hairs beneath a clump of hairs basally above. Gnathos slender, almost straight in lateral view, and bare. Upper portion of clasper membranous and thickly covered with long hairs, separated from lower portion except basally; lower portion sclerotized and with a pair of long spinelike processes that curve toward each other, the inner (and more basal) process being slightly

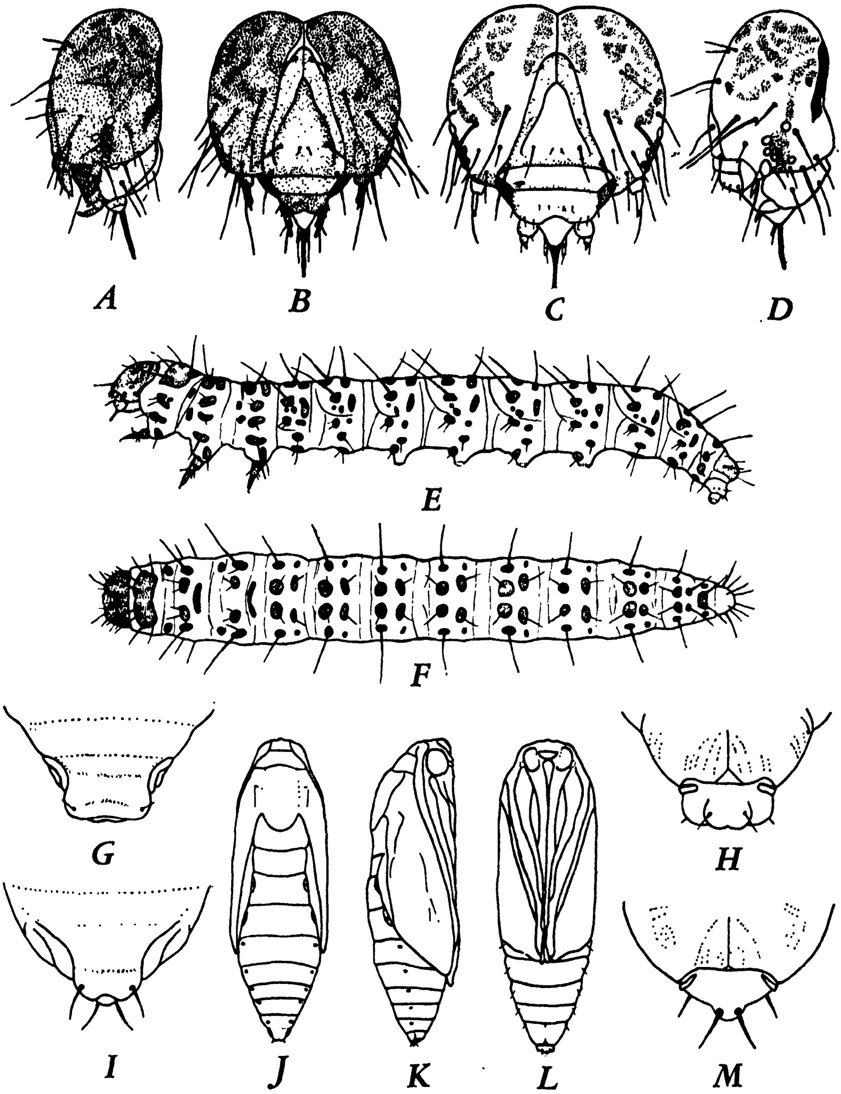

Fig. 4.—*A, B, E–H, J–L, Crambus bonifatellus: A, B,* lateral and frontal views of head of mature larva; *E, F,* lateral and dorsal views of mature larva; *G, H,* dorsal and ventral views of abdominal apex of pupa; *J, K, L,* dorsal, lateral, and ventral views of pupa. *C, D, I, M, C. sperryellus: C, D,* frontal and lateral views of head of mature larva; *I, M,* dorsal and ventral views of abdominal apex of pupa.

the longer and more slender at its base; inner edge of upper process thickly covered with long hairs along its thickened basal half. Aedeagus somewhat longer than claspers, stout, pointed apically, and without hook.

Egg.—(Fig. 3) Elliptical, bluntly rounded at apexes; ornamented with about 35 longitudinal rows of depressed hexagonal plates, some rows incomplete, detail of plates and ribs becoming obscure toward apexes of egg. Color ranging from lemon yellow at deposition through orange to purplish shortly before eclosion. Average length 1.58 mm; average width 1.35 mm.

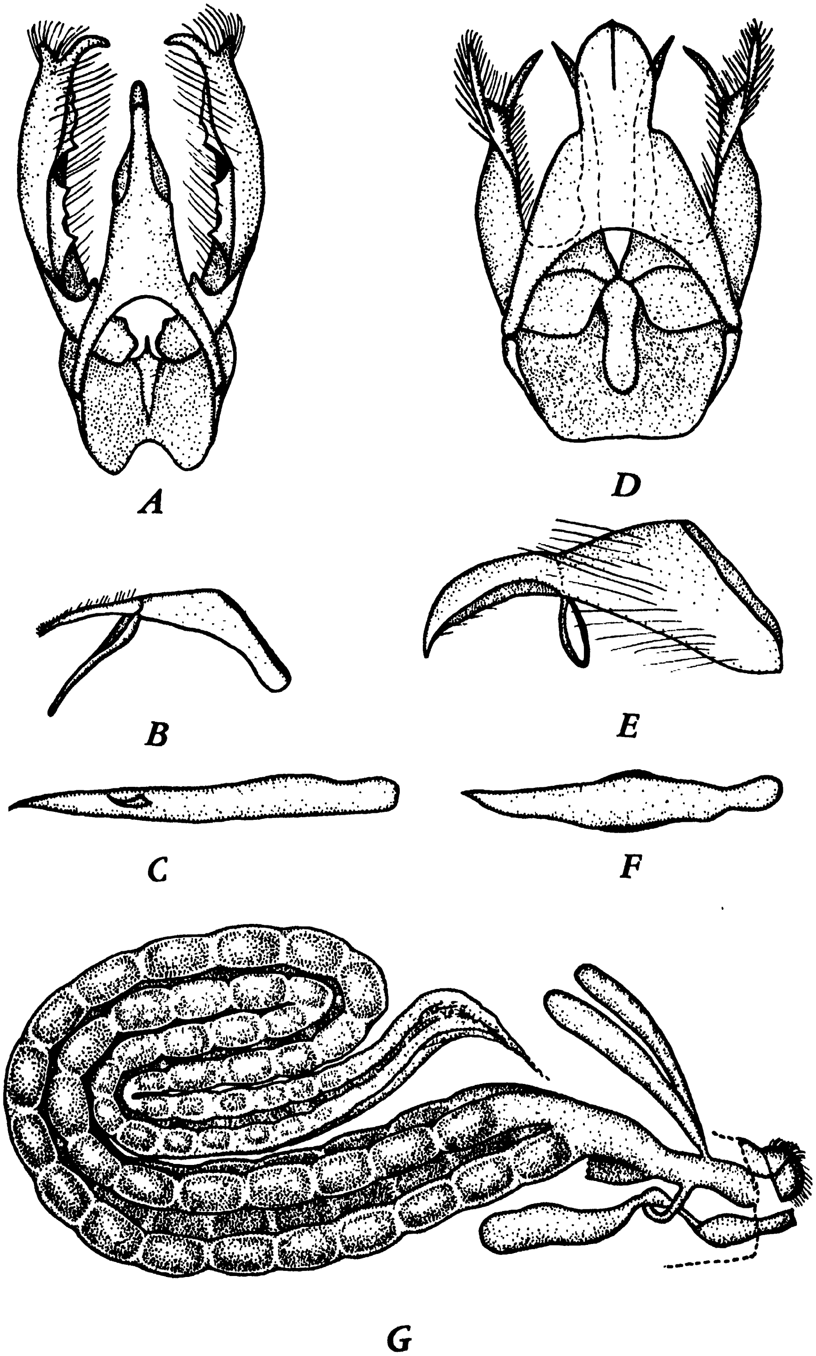

Fig. 5.—*A–C,* Male genitalia of *Crambus sperryellus: A,* parts in natural position, dorsal; *B,* tegumen, lateral; *C,* aedeagus, dorsal. *D–F,* Corresponding male genitalia of *C. bonifatellus. G,* Female genital system of *C. bonifatellus,* lateral (one ovary removed).

The color range through which the egg passes is exceptionally striking. During a 5-day incubation period, the change proceeds as follows: The pure lemon yellow at the time of deposition turns after one day to salmon pink. On the third day a small black eyespot becomes visible near one end, and on the succeeding day the dark larval head and prothorax can be seen taking up the anterior third of the egg. At this time the color is somewhat deeper, and on the following or fifth day it takes on a bluish to purplish cast heralding eclosion.

First Instar.—Head dark brown, legs and caudal plate dusky gray, pronotal plate grayish brown, remainder of body pale orange yellow, with inconspicuous spots. Average body length shortly before molting 1.2 mm; average breadth of head 1.235 mm.

Second Instar.—Similar to preceding instar except for larger size, grayish ground color, appearance of two spots below pronotal shield, and faintly outlined spots around the bases of the body bristles. Average body length shortly before molting 2 mm; average breadth of head 1.336 mm.

Third Instar.—Not appreciably different from the second instar except in size. Average body length shortly before molting 3.5 mm; average breadth of head 1.553 mm.

Fourth Instar.—Body spots faint but distinct; spiracles dark and distinct; otherwise similar to third instar except in size. Average body length shortly before molting 5.5 mm; average breadth of head 1.691 mm.

Fifth Instar.—Body spots well developed, but only moderately dark; body ground color grayish white; head brown to dark brown, with darker markings faintly visible on its upper half; caudal and pronotal plates sprinkled with small dark spots. Average body length shortly before molting 9 mm; average breadth of head 1.936 mm.

Sixth Instar.—(Fig. 2, *C*; fig. 4, *A, B, E,* and *F*) Similar to preceding instar except for greater size and darker body spots. Average body length shortly before pupation 15 mm; average breadth of head 1.307 mm.

Seventh and Following Instars.—The seventh and following instars, when they occur, resemble the sixth. Under certain conditions a larva may go through as many as 10 instars; but ordinarily pupation takes place at the end of the sixth.

Pupa.—(Fig. 4, *G, H, J–L*) Pale yellow when first formed, but gradually deepening toward brown in color, eyespot becoming almost black shortly before emergence. Oval spiracles present on abdominal segments 3 to 9, that on 9 faint, those on 3 and 4 enclosed by semicircular lines from hind-wing-case margins, those on 8 and 9 particularly elevated. Caudal process with a roughly trapezoid-shaped dorsal plate, bordered laterally by a depressed and irregularly outlined area; apical margin of dorsal plate with a slightly convex, nearly regular outline; apical angles each with a dorsally directed bristle. Ventrally this process is about as long as broad and bears 2 posteriorly directed bristles, originating subapically close to the middle. Average length of pupa about 8 mm.

TECHNICAL DESCRIPTION OF STAGES OF CRAMBUS SPERRYELLUS

Adult.—(Fig. 6) Head, palpi above, antennae, forewings beneath, and most of legs, brownish tinted with gold; abdomen above, buff and silver; undersides of palpi, thorax, hind wings, and abdomen silvery white; thorax above, bright golden. Forewing basal to submarginal line bright golden with a broad streak of shining silver, narrowly edged in dark brown and tapered to a point shortly before the submarginal line of dark brown, which is outwardly edged with silver; apex of wing with an indistinctly outlined silver spot and separated from the large silver stripe by a creamy white spot; 5 posterior veins ornamented with narrow black spots between submarginal line and brown marginal line; fringe brown and silver. Hind wing silvery gray with a darker edge and silver fringe. Wing expanse, female 21 to 24 mm, male 18 to 21 mm. For the male genitalia, see also figure 5, *A, B, C.* Tegumen slender. Uncus somewhat petiolate, shorter than gnathos, apically rounded and with short backward-pointing bristles that become longer and forward-pointing basally. Gnathos slender, bare, and almost straight in lateral view. Upper portion of clasper membranous, covered with long hairs, and attached for almost its whole length to lower portion; lower portion sclerotized, inner margin sparsely covered with bristles but conspicuously serrate, with about six inward-pointing teeth distributed over the middle two thirds; free

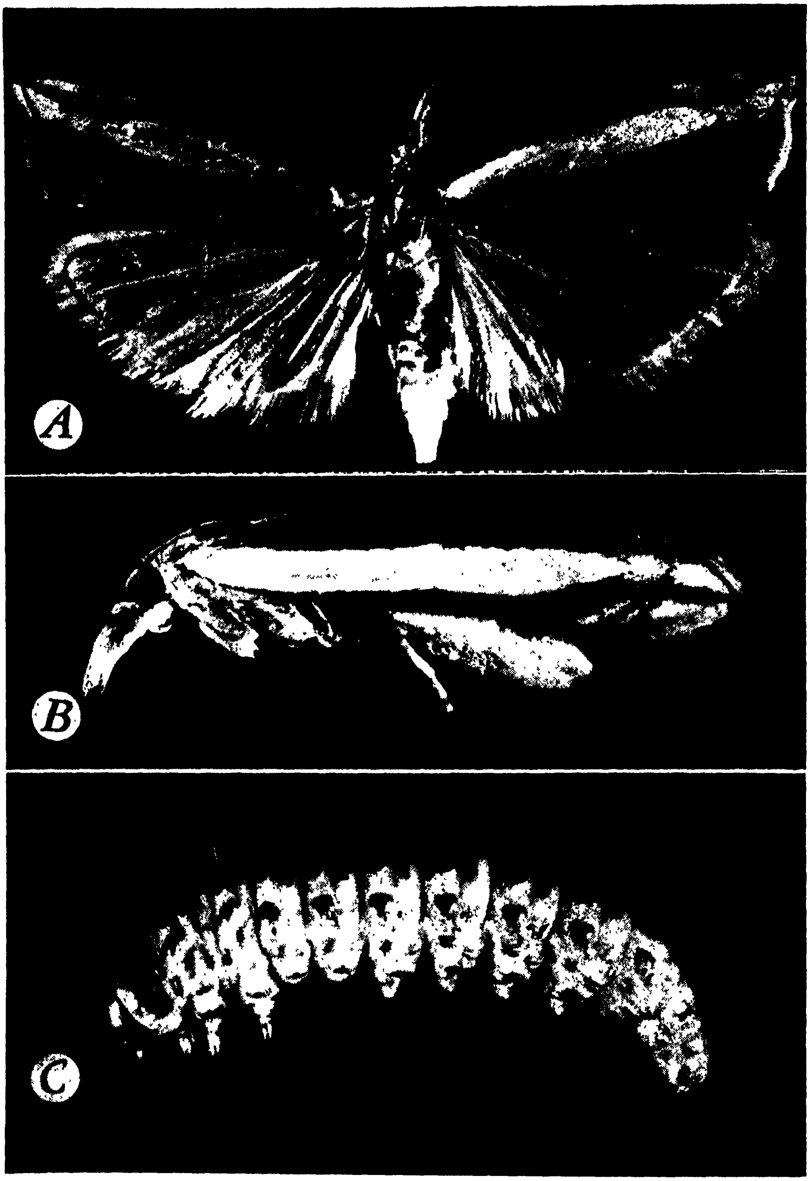

Fig. 6.—*Crambus sperryellus* Klots: *A* and *B*, adult female; *C*, seventh-instar larva.

end slender, curved, and narrowly rounded apically. Aedeagus tapering to a sharply pointed apex, with a large curved lateral hooklike process situated at apical third.

Egg.—Similar to that of *Crambus bonifatellus* in structure, but different in color range during the incubation period. With a 6-day incubation period the lemon-yellow eggs after one day change gradually through pale salmon pink to cherry red near the end of the fourth day. Eyespots are discernible 12 hours later, and the nearly developed larva is visible within its shell after 5½ days.

First Instar.—Head dark brown, legs and caudal plate dusky gray, pronotal plate somewhat darker, remainder of body pale orange yellow, body spots inconspicuous. Average body length shortly before molting 1.2 mm; average breadth of head 0.242 mm.

Second Instar.—Not appreciably different from first instar except for the more grayish ground color, larger size, and proportionately smaller head. Average body length shortly before molting 2 mm; average breadth of head 0.351 mm.

Third Instar.—Not appreciably different from second instar except for the more mottled body color and larger size. Average body length shortly before molting 3.5 mm; average breadth of head 0.595 mm.

Fourth Instar.—Body mottled as in previous instar; but spots not distinct, except slightly so on thorax; stigmatal spot black on prothorax, less distinct elsewhere. Head dark brown, with light-brown areas above the eyes and in the region of the frons; legs and pronotal plate dark grayish brown. Average body length shortly before molting 5.5 mm; average breadth of head 0.788 mm.

Fifth Instar.—Body grayish white with distinct grayish-brown spots; head brownish yellow, ornamented on its upper half with flocculent brownish spots; eye region, mandibles, and lateral margin of the head above the eyes, black; caudal plate gray and covered with about 25 small scattered dark spots; pronotal plate with an equal number of similar spots; stigmatal spots black. Average length of the body shortly before molting 9 mm; average breadth of head 1.018 mm.

Sixth Instar.—Similar to fifth instar except for the pale, more whitish body ground color, the paler, more yellowish head ground color, and the larger size. Average length of body shortly before molting 15 mm; average breadth of head 1.325 mm.

Seventh Instar.—(Fig. 4, *C, D*; 6, *C*) Similar to sixth instar except for greater size and ordinarily lighter ground color of the head. Average length of body shortly before molting 15 mm; average breadth of head 1.708 mm.

Eighth and Following Instars.—Approximating seventh instar in size and markings. Pupation ordinarily takes place after the seventh instar, but under some conditions as many as 9 instars have been observed.

Summary of Differences in Appearance of Crambus Bonifatellus and Crambus Sperryellus.—The adult *Crambus bonifatellus,* with its spotted gray forewings, differs from *C. sperryellus,* which has silver-striped forewings. Ample distinction is also afforded by the male genitalia (fig. 5, *A–F*). The eggs of the two species appear identical in structure, but differ slightly in color range during incubation. The larvae are practically indistinguishable in the first three instars; but in the fourth and following instars there is a distinct difference in head pigmentation. In *C. sperryellus* the dark spots on the upper half of the head contrast strongly with the light background; but in *C. bonifatellus* the spots are indistinct, and the ground color is usually brown or nearly black. The pupae of the two species have distinctive caudal processes. That of *C. bonifatellus* is blunt (fig. 4, *G, H*), whereas that of *C. sperryellus* is roundly pointed (fig. 4, *I, M*).

LIFE HISTORY AND HABITS OF CRAMBUS BONIFATELLUS

Adult.—Judging from laboratory specimens, emergence from the pupa takes place in late afternoon or evening, and females cannot lay fertile eggs until two nights later. Copulation has not been noted; it apparently does not occur in flight, for the writer has observed and captured thousands of flying specimens throughout the season without seeing any copulating pairs.

Neither sex appears to be attracted to flowers nor to take any food under natural conditions. In captivity, however, the moths eagerly drink water or sugar solution.

The average life span of *Crambus bonifatellus* collected in the field was 4.2 days at 75° F under natural light conditions. When the temperature was lowered to 59° and 41°, longevity was increased to an average of 13.6 and 14.0 days respectively.

Perhaps the most characteristic action of lawn moths is their oviposition flight. Just at dusk during the flight season the females begin to leave their hiding places in grass or shrubbery and appear over lawns. For about an hour they maintain a hovering, undulating flight, during which they are rarely more than a foot above the grass. In the laboratory moths were observed to drop their eggs as they fluttered in glass jars. The eggs are not adhesive and are dropped either in flight or at rest.

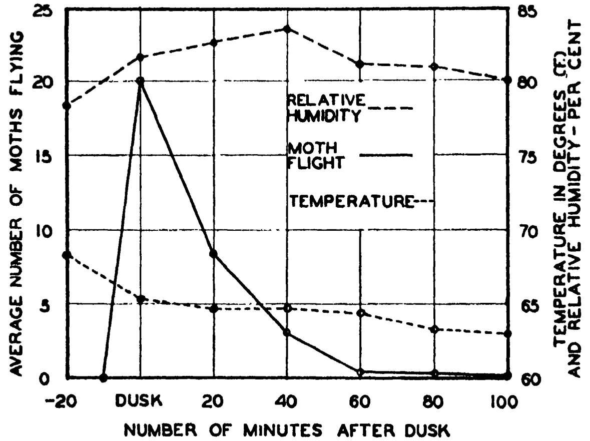

Fig. 7.—Flight of *Crambus bonifatellus* in relation to time of day, temperature, and relative humidity, computed from average figures of weekly observations, June 17 to September 16, 1941, on a Los Angeles lawn. An area of 1,000 square feet was taken as a unit.

The oviposition flight appears to depend chiefly on natural light conditions. Weekly observations during the summer of 1941 revealed no correlation between the time or extent of the flight and the temperature or humidity. Figure 7 summarizes these data. The flight reaches a peak at dusk and falls off rapidly thereafter. Twenty minutes after dusk it has decreased by approximately half, and 40 minutes after dusk it has again decreased by half. Except for a few stragglers it lasts only an hour. In the latter part of this period, moths usually flutter above shrubbery at the edge of the lawn. These are predominantly males, which rarely fly in the open. During oviposition the moths invariably concentrate where the grass is most lush. Similarly, largest numbers in any one locality are usually found over the new and tender lawns. This habit may partially explain the disproportionate damage to the lush bent grass in areas where Bermuda or bluegrass predominates.

Though both sexes are mildly attracted to lights, the attraction appears to be ineffective until oviposition is nearly over. Even when moths are abundant, relatively few gather about street or porch lights.

Oviposition data from moths collected in the field are summarized in table 3, and presented graphically in figure 8. Eggs were counted for 100 moths kept at about 75° F and exposed to natural light in the laboratory. Relatively few eggs were laid on the night of capture, probably because of the upset to the moth's routine. The largest number were laid the next night after capture, with an average of 74.4. The average total number per moth was 187.3. Of the 100 moths, 18 per cent laid more than 300 eggs; and one moth produced 587 over a week's period, with 211 in a single night.

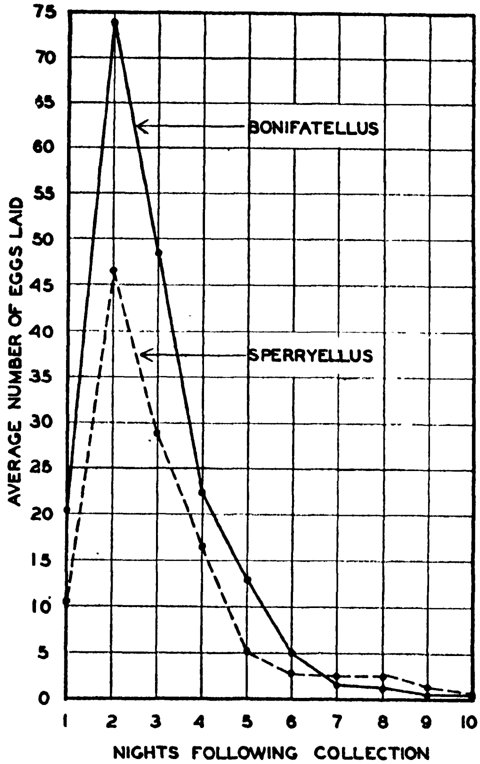

Fig. 8.—Comparison of the oviposition of *Crambus bonifatellus* and *C. sperryellus* collected in the field. One hundred specimens each were kept in shell vials at room temperature (73°–77° F) under natural light conditions.

Several females collected in the field were dissected for a study of the reproductive system (fig. 5, *G*). Dissections were also made of moths that had laid a full quota. In these, only 1 or 2 well-formed eggs were found in the ovarioles; and the ovaries appeared to be degenerating.

Certain effects of temperature, light, and food on egg production were studied. Moths kept in the dark at constant temperatures laid an average of 6 eggs at 41° F, 170 at 59°, and 102 at 75°. Those in natural light at about 75° laid an average of 187 eggs. Apparently, then, natural light conditions favor egg production, and the total number of eggs is not much affected by temperatures as low as 59°.

Egg.—The incubation period varies according to the temperature. Eggs kept at 41° F did not develop, although they remained viable for 74 days. The incubation period at 59° was 15 days; at 75° it was 4.5; and at 86° or 98° it was 3.5. These data, as related to egg coloration, are given in table 4.

In rearing experiments, a high percentage of the eggs from females collected in the field proved to be fertile. Of 1,407 eggs laid by 14 moths captured at various times during the summer, 1,400, or 99.5 per cent, hatched. Evidently, however, a certain amount of moisture is required during incubation. More than 1,000 eggs kept in a cotton-stoppered shell vial without additional moisture produced only 5 larvae. At the other extreme, only 6 larvae emerged from 25 eggs completely immersed in water during incubation. Practically 100 per cent hatched when the eggs were kept on moist blotter paper, as in the records mentioned at the beginning of the paragraph, or when they were placed on dry blotter paper and watered daily. In another test, of 1,250 eggs that were floated on water throughout incubation, 98 per cent hatched.

TABLE 3

OVIPOSITION DATA ON 100 MOTHS EACH OF *Crambus bonifatellus* AND *C. sperryellus*, COLLECTED IN THE FIELD*

Species	Average number of eggs laid	Number of moths ovipositing in each range given					
		1-100 eggs	101-200 eggs	201-300 eggs	301-400 eggs	401-500 eggs	501-600 eggs
	eggs	*moths*	*moths*	*moths*	*moths*	*moths*	*moths*
C. bonifatellus..................	187	28	28	26	14	3	1†
C. sperryellus..................	118	45	37	13	4	0	1‡

* The moths were kept at a laboratory temperature of 73° to 77° F.
† This specimen laid 587 eggs in 6 days.
‡ This specimen laid 528 eggs in 8 days.

TABLE 4

RELATION OF CHANGES IN EGG COLOR TO TIME AND TEMPERATURE IN THE INCUBATION PERIOD OF *Crambus bonifatellus*

Degrees Fahrenheit (constant temperature)	Time in stages of incubation*					Total incubation period
	Yellow	Pale pink	Salmon pink to deep pink	Eyespots discernible	Nearly mature embryo visible	
	days	*days*	*days*	*days*	*days*	*days*
41..........................	8.0	12.0	74.0†	....	...	....
59..........................	2.0	5.0	2.5	4.0	1.5	15.0
75..........................	1.0	0.5	1.5	1.0	0.5	4.5
86..........................	0.75	0.5	1.0	0.75	0.5	3.5
98..........................	0.75	0.5	1.0	0.75	0.5	3.5

* Based on an average of 3 tests of 100 eggs each for each temperature.
† Eggs shriveled and molded at this time without developing further.

Eggs were obtained from several moths reared in the laboratory. As would be expected, virgin females produced infertile eggs, which shriveled a few days later without changing color. Attempts were made to insure fertilization by mating virgin females with freshly emerged males. Although copulation was not observed, one female did lay fertile eggs 2 days after being confined with a male.

Larva.—Previous authors have commented on the difficulty of rearing *Crambus* in the laboratory. It is not easy to provide the requirements of fresh grass, moderately high humidity without condensed moisture, and freedom from mold, while closely observing the life history. In consequence, several methods of rearing were tried before a satisfactory one was developed. At first individual larvae were placed in small, slender glass vials stoppered with cotton. This method proved impractical because of the difficulty of maintaining proper humidity and cleanliness. Second, transplanted grass in small flowerpots was artifically infested with eggs, and the larval development was followed as closely as possible. Accurate observations could not, however, be made; and only a general idea of the life history and the amount of feeding was obtained. In the method finally employed, the bottoms of small stender dishes were lined with several layers of close-fitting damp blotter paper. Fifteen newly hatched larvae were placed in each dish; as they increased in size, the number was reduced to prevent overcrowding. Each dish was examined daily, thoroughly cleaned, and supplied with fresh-cut grass. Enough water was added to keep the blotter lining damp, and the dishes were kept in the dark to approximate the natural conditions.

TABLE 5

LARVAL HEAD-BREADTH MEASUREMENTS* OF *Crambus bonifatellus* AND *C. sperryellus* COMPARED WITH THE THEORETICAL MEASUREMENTS COMPUTED ACCORDING TO DYAR'S LAW

Instar	*Crambus bonifatellus*		*Crambus sperryellus*	
	Actual breadth	Theoretical breadth, factor = 1.43	Actual breadth	Theoretical breadth, factor = 1.43
	mm	*mm*	*mm*	*mm*
1	0.235	0.235	0.242	0.242
2	0.364	0.336	0.351	0.351
3	0.553	0.481	0.595	0.493
4	0.691	0.688	0.788	0 705
5	0.936	0.984	1.018	1 008
6	1.307	1.401	1.325	1.441
7	.....	.....	1.708	2.060

* Measurements based on an average of 10 specimens each reared in the laboratory.

Records of the instars were obtained by noting the characteristically swollen premolt condition and by collecting the cast head capsules. Unlike *Crambus praefectellus* Zinck., as recorded by Ainslee (1923*b*), the larvae consumed neither these capsules nor the eggshells. Table 5 gives measurements of the cast head capsules. The increase in head size roughly follows a geometrical progression with a factor of 1.43.

Figure 9 shows the types of damage done by the different larval stages. The first two instars work chiefly as skeletonizers of the grass blades. Usually they begin feeding on the upper surface of the leaf, but avoid the veins. All stages spin silken shelters camouflaged with bits of grass and excrement. In the first two instars these shelters must be built on a single leaf blade. Older larvae may utilize several blades of grass in constructing their homes. When disturbed, young larvae readily drop to the ground by suspending themselves on an anal thread. Larvae of all sizes can curl into a compact ball when danger threatens; but after the third molt they more often depend on their agility for escape. They crawl backward as rapidly as forward and, if touched along the side of the body by any moving object, will thresh wildly about. In this respect *Crambus bonifatellus* is much more sensitive than *C. sperryellus.*

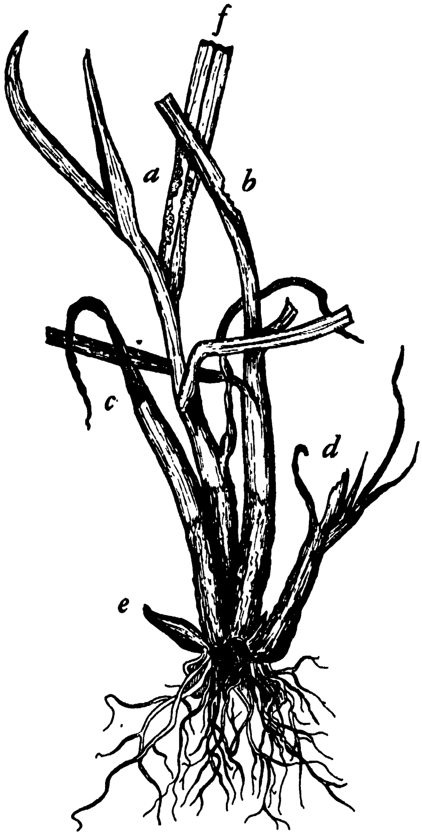

Fig. 9.—Typical injury to bent grass by *Crambus* larvae; *a,* skeletonizing by first-instar larvae; *b,* leaf-notching by third- or fourth-instar larvae; *c–e,* young shoots fed upon by larvae; *f,* entire end of blade removed by mature larva.

Third-instar and older larvae notch the edges of grass and often sever the blades completely, afterwards drawing them into the shelters to be consumed at leisure. Though this latter process can occasionally be seen in the daytime, feeding is much more evident at night. The writer observed night feeding between 8:00 and 10:00 p.m., September 2, 1940, on a heavily infested lawn. With the aid of a flashlight he saw the larvae cutting grass and drawing it into their burrows. A telltale waving of a grass blade gave the first indication of a webworm's presence. A direct ray of light or the slightest movement of the observer halted the work up to several yards away. The larvae particularly relish the fresh tender grass shoots and will chew these back as rapidly as they appear. They also attack the greener portions of the crown, but, contrary to popular opinion, they do not molest the grass roots. This observation agrees with those made by Ainslee (1923*a*, 1923*b*, 1930) on *Crambus mutabilis* Clemens, *C. praefectellus,* and *C. teterrellus* Zinck. and by Jewett (1939) on *C. mutabilis, C. teterrellus,* and *C. trisectus* Walker. Fresh sod-webworm frass is invariably a bright grass green, which would not be its color if the larvae were feeding on the nearly colorless grass roots. To obtain some definite information on this point, fourth- and fifth-instar larvae were placed in dishes containing a few bluegrass blades and crowns, and a large quantity of fresh grass roots. The grass blades were eaten at once, and also the greener portions of the crown. After 3 days all green material had been consumed. Although fresh roots were provided daily, no evidence of feeding on them could be discerned even after several days' starvation.

Well-grown larvae consume considerable grass. According to records made at 75° F, fifth-instar larvae eat about twice their weight of bluegrass daily.

Molting takes place within the protecting confines of a silken shelter. A premolt period beginning about 12 hours before ecdysis (at 75° F) is marked by a swollen appearance, especially in the neck region. At this time the larva is quiescent, takes no food, and cannot crawl freely.

TABLE 6

LENGTH OF TIME IN EACH PERIOD IN THE LIFE CYCLE OF *Crambus bonifatellus* AT DIFFERENT TEMPERATURES WITH BLUEGRASS BLADES AS LARVAL FOOD

Degrees Fahrenheit	Egg			Larva			Pupa			Total		
	Minimum	Average*	Maximum	Minimum	Average*	Maximum	Minimum	Average*	Maximum	Minimum	Average*	Maximum
	days	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*
41	....†	...†	...†	....	....	....	....	....	.	....	...	...
59	14.0	15.0	16.0	82.0	91.7	120.0‡	27.0	27.0	27.0	123.0	133.7	163.0
75	4.5	4.5	4.5	18.0	23.7§	42.0	6.0	7.7	11.0	28.5	35.9	67.5
86	3.5	3.5	3.5	17.0	22.7	30.0	7.0	7.0	7.0	27.5	33.2	40.5
98	3.5	3.5	3.5	....†	....†	....†	....	....	....	....	....	....

* Averages were based on 200 eggs at each temperature; 9 larvae at 59° F, 7 larvae at 86°, 75 larvae at 75°, 1 pupa at 59°, 63 pupae at 75°, 4 pupae at 86°.

† Specimens died before completing this period.

‡ Two larvae that died before pupation had lived 141 and 149 days.

§ Two specimens reared out of doors at temperatures of 55° to 87° F completed the larval period in 31 and 33 days.

TABLE 7

RELATION OF CHANGES IN EGG COLOR TO TIME AND TEMPERATURE IN THE INCUBATION PERIOD OF *Crambus sperryellus*

Temperature	Length of time in stages of incubation*					Total incubation period
	Yellow	Pale to deep salmon pink	Orange to cherry red	Eyespots discernible	Nearly mature embryo visible	
Degrees F	*days*	*days*	*days*	*days*	*days*	*days*
41	22.0	72.0†	....	....	....	....
59	3.0	2.0	9.0	5.0	2.0	21.0
75	1.0	0.5	2.5	1.0	1.0	6.0
86	1.0	0.25	1.5	1.0	0.75	4.5
98	0.75	0.25	1.25	0.75	0.5	3.5

* Based on an average of 2 tests of 100 eggs each for each temperature.

† Eggs shriveled and molded at this time without developing further.

The duration of the various larval stages varies with the temperature. At approximately 75° F, with bluegrass as food, there are normally six larval instars, the first five of which average 3½ days each in length. The sixth instar averages 6 days. Table 6 gives figures on the complete larval period of *Crambus bonifatellus* reared in the laboratory at various constant temperatures. At 41° and 98° no larvae were reared to maturity. At 86° and 75° the larvae showed a pronounced tendency toward cannibalism, which was not at all evident among those reared at 59°.

Pupa.—After the last-stage larva has stopped feeding, it reinforces the silken larval shelter and pupates within it. Transformation is slow; the larva lies quiescent for 2 or 3 days before quickly shedding its skin. After this process the split head capsule and a bit of crumpled skin are all that remains of the larval integument. The pupa is usually found within the top inch of soil. Figure 10 shows a piece of sod cut to expose the cocoon in its natural position. The length of the pupal stage depends upon the temperature. Laboratory specimens emerged after 27 days at 59° F, 6 to 11 days (average 7.7) at 75°, and 7 days at 86°. The pupal period was practically the same for both sexes at 75°, the only temperature at which an appreciable number of pupae were available. The average pupal period for 33 females was 7.8 days; for 30 males, 7.6.

Fig. 10.—Section of sod cut to expose cocoon and pupa of *Crambus bonifatellus*. (Natural size.)

LIFE HISTORY AND HABITS OF CRAMBUS SPERRYELLUS

Apparently the habits and general life history are much the same in *Crambus sperryellus* as in *C. bonifatellus*. Since the latter has been discussed in detail, only the differences between the two need now be pointed out.

Adult.—*Crambus sperryellus* lived longer in captivity than *C. bonifatellus* at all temperatures used except 41° F. It laid far fewer eggs, however—especially at 59°, when an average of only 15 eggs were laid as compared with 170 for *C. bonifatellus.*

Daily egg counts were made of 100 moths collected in the field and kept at about 75° F under natural light conditions in the laboratory. Figure 8 is a graph of the results. The curve parallels that of *Crambus bonifatellus* closely, but at lower levels.

The first night there was an average of 11.2 eggs. The second night the largest number was laid, namely 46.7. The average total number was 118, as opposed to 187 for *C. bonifatellus.* Of the 100 moths, only 5 laid more than 300 eggs (310, 310, 311, 323, 528); and only 13 laid from 200 to 300. Table 3 (p. 282) summarizes these data.

Egg.—Table 7 correlates the changes of egg color with time and temperature during incubation. Judging from these data, under natural summer lawn temperatures of about 55° to 85° F the incubation period of *C. sperryellus* would be 2 or 3 days longer than that of *C. bonifatellus* (see also table 8). The fertility of *C. sperryellus* eggs is indicated by the 100 per cent hatch of 420 eggs laid by 6 females collected in the field.

TABLE 8

LENGTH OF TIME IN EACH PERIOD IN THE LIFE CYCLE OF *Crambus sperryellus* AT DIFFERENT TEMPERATURES WITH BLUEGRASS BLADES AS LARVAL FOOD

Temperature	Egg			Larva			Pupa			Total		
	Minimum	Average*	Maximum	Minimum	Average*	Maximum	Minimum	Average*	Maximum	Minimum	Average*	Maximum
Deg. F	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*
41......	. .	..	..	....	....	....	....	. ..	..	. .	....	....
59......	21.0	21.1	22.0	. †	.. †	†	...	.	..		..	..
75	5.5	6.0	6.5	27.0	37.6	54.0	10.0	11.2	13.0	42.5	54.8	73.5
86.. ...	4.5	4.5	... †	. †	. †	....	....	....	....	....	..	....
98......	3.5	3.5	. †	†	....†	..	....	. ..	....	. .	.	.

* Averages were based on 200 eggs at each temperature; 12 larvae and 12 pupae at 75°.
† Specimens died before completing this period.

Larva.—For some unknown reason *Crambus sperryellus* proved exceedingly difficult to rear in the laboratory. At 59° F an entire lot of 30 larvae died in the first instar; at 75° only 12 out of 200 matured successfully; at 86° 2 larvae out of 30 reached the seventh instar and then died; and at 98° the sixth and seventh instars were attained by 1 larva each out of 30, but these died before pupation. In these experiments, Kentucky bluegrass served as food. Various other grasses were substituted without significantly better results (table 1, p. 270). At about 75° the larval period averaged 37.6 days (table 8), as opposed to 23.7 days for *C. bonifatellus.*

Besides appearing bulkier, the larvae were much more sluggish in all stages than *Crambus bonifatellus* and constructed their shelters more carefully. Ordinarily *Crambus sperryellus* had 7 instars, but sometimes an eighth and even a ninth.

Table 5 (p. 283) presents measurements of the cast head capsules. As may be seen by comparing the columns on actual and on theoretical breadth, the increase in head size roughly follows a geometrical progression with a factor of 1.43.

Pupa.—Specimens reared in the laboratory at about 75° F emerged in 10 to 13 days, with an average of 11.2, as compared with a corresponding period of 7.7 days for *Crambus bonifatellus* (table 8).

SEASONAL HISTORY

In contrast with the eastern North American species, whose seasonal histories are characterized by definite summer broods, the California lawn-infesting *Crambus* appear to breed continuously from May through October. The explanation may be that the California species have an economic history

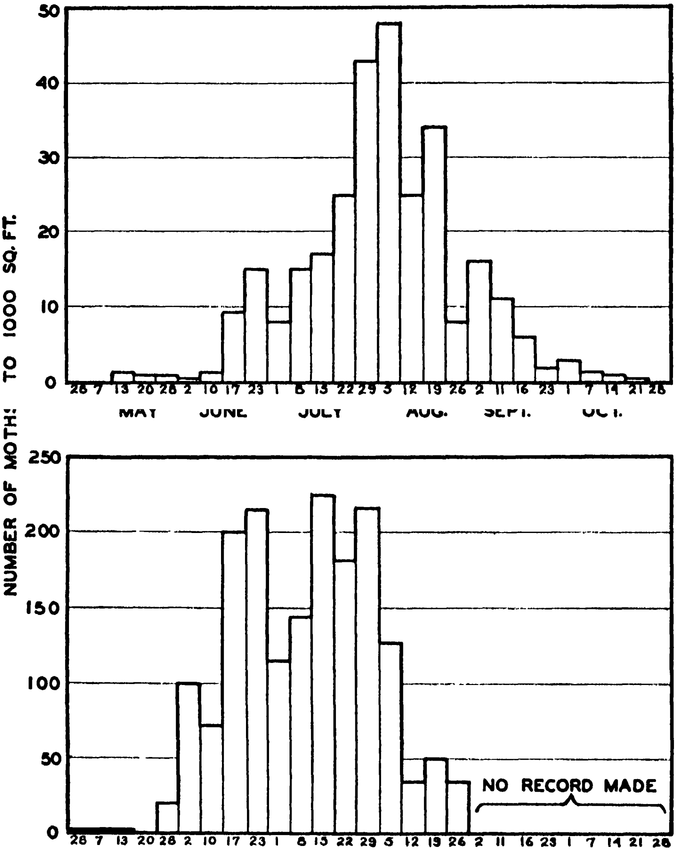

Fig. 11.—Comparison of the weekly flight record of *Crambus bonifatellus* over two different lawns in Los Angeles, 1941. Lower graph based on a more heavily infested lawn and drawn to a smaller scale.

only on artificially watered lawn grass, which provides a constant supply of fresh food under relatively moist conditions. The eastern species, on the other hand, which attack primarily unirrigated grassland or grain crops, depend on spring and summer rains for moisture. According to Ainslee (1930), "... a continuous growth of young, tender grass is essential to the welfare of the larvae [of *C. teterellus* Zinck.], especially while they are small, and the mortality is very great among larvae hatching when the ground is dry and hot and the grass rough and closely cropped." Ainslee states further: "Apparently the larvae approaching or reaching full development during such a dry period lie quiescent for some time, instead of pupating immediately, but resume their development when supplied with moisture." Infestations of well-kept turf in eastern North America have been largely incidental to those on unirrigated land, and no seasonal-history studies have been reported from turf alone.

According to field notes and collecting records made in 1939, 1940, and 1941 in the Los Angeles area, adult moths were fairly common from the middle of May to the middle of October. Early-season records for *Crambus sperryellus* were April 20, 1939; April 11, 1940; and March 26, 1941. For *C. bonifatellus* early records were April 28, 1939; April 13, 1940; March 26, 1941; and January 23, 1942 (one female at light). Late-season records for *C. sperryellus* were October 15, 1938; October 22, 1939; October 19, 1940; and October 21, 1941. For *C. bonifatellus* late records were October 15, 1938; November 1, 1939; October 19, 1940; and November 17, 1941. The early records were of females, and all the late ones of males. The following are early- and late-season flight records from sections outside the Los Angeles area.

Crambus sperryellus: San Diego, October 27, 1913 (W. S. Wright); San Diego, April 3, 1941 (R. M. Bohart); Potholes, Imperial County, April 7, 1923 (E. P. Van Duzee); Whittier, March 17, 1940 (R. H. Smith); Sacramento, November 20, 1928 (H. H. Keifer); Mill Valley, Marin County, October 24, 1926 (E. P. Van Duzee).

Crambus bonifatellus: San Diego, March 4, 1916 (W. S. Wright), April 3, 1941 (R. M. Bohart); Riverside, March 8, 1941 (R. H. Smith); Lompoc, March 28, 1941 (R. M. Bohart); San Francisco, April and October (R. M. Bohart); Berkeley, October (R. M. Bohart).

During the 1941 season, *Crambus bonifatellus* moth flight was observed regularly on two lawns in West Los Angeles. These data are presented graphically in figure 11. On one lawn the first moth was seen May 13, and the last specimen on October 21; but most of the flight occurred during 3 months between June 17 and September 16. Heaviest flights observed were about the middle of this period, on July 29 and August 5. On the second lawn the first flight noted was on April 28, and most of the flights came between June 2 and August 26. No single peak was noted on the second lawn. Records of 200 or more moths to 1,000 square feet were made on June 17, June 23, July 15, and July 29.

On the basis of field observations, particularly on the reinfestation of treated lawns, and laboratory rearing data (tables 6 and 8, pages 285 and 287), it seems that in the Los Angeles area *Crambus bonifatellus* completes four generations a year and *C. sperryellus* only three. Apparently, however, the generations overlap in both species.

The California lawn moths seem to overwinter in the larval stage only. This agrees with published findings on other species of *Crambus* in eastern North America. In California the writer brought sixth-instar larvae of *C. bonifatellus* and *C. sperryellus* to the surface of lawns with pyrethrum solution in November, December, January, February, and March of 1940 and 1941. Specimens dissected from sod during the winter were found in tubelike shelters resembling those where pupation takes place and somewhat more tightly constructed than larval shelters made during the summer. The overwintering shelters probably serve as protection against natural enemies as well as against climatic conditions. In the opinion of Ainslee (1923*a*), similar shelters of

Crambus mutabilis may be used primarily to keep the larvae dry, not to protect them from cold.

The size of the overwintering population probably varies greatly, depending on the activity of natural enemies and upon the climate. Pyrethrum tests were made February 3, 1942, on a lawn that had been the subject of flight records the previous summer (fig. 11, upper graph). Twenty plots, 10 square feet in area, were selected at random over 3,000 square feet. Six of these plots harbored 1 mature *Crambus bonifatellus* larva each. From this test it was estimated that 30 larvae were present to 1,000 square feet, as compared with the average of 150 larvae estimated for the same area on August 1, 1941.

TABLE 9

PARASITES RECORDED FROM NORTH AMERICAN SPECIES OF *Crambus*

Parasite	Host species of *Crambus*	Locality	Reference
	Ichneumonidae		
Limneria argentifrons Cress.	*zeelus* Clemens	Indiana	Anon. (1890)
Cryptus mundus Prov.	*vulgivagellus* Clemens		Anon. (1890)
Lissonota frigida Cress.	*vulgivagellus* Clemens	New York Kansas	Anon. (1891) Miller (1940)
Angitia obscura Cress. *Pimpla annulipes* Brullé	*trisectus* Walker	Indiana	Ainslee (1927)
Cymodusa mississippiensis Ashm.	undet. sp.	Indiana	Ainslee (1927)
Zele nigriceps Riley	*zeelus* Clemens	Indiana	Anon. (1890)
	Braconidae		
Macrocentrus crambivorus Vier.	*mutabilis* Clemens	Tennessee	Ainslee (1923*a*)
Apanteles crambi Weed	*mutabilis* Clemens	South Dakota Tennessee	Ainslee (1923*a*) Ainslee (1923*a*)
Apanteles sp.	undet. spp.	Ontario, Canada	Stirret and Arnott (1932)
Apanteles sp.	undet. spp.	Eastern U.S.	Noble (1932)
Apanteles sp.	*bonifatellus* (Hulst) *sperryellus* Klots	California California	Original records Original records
Orgilus detectiformis Vier.	*trisectus* Walker	Indiana	Ainslee (1927)
Orgilus sp.	*bonifatellus* (Hulst) *sperryellus* Klots	California California	Original records Original records
	Tachinidae		
Phorocera claripennis Macq.	*mutabilis* Clemens undet. spp.	Indiana Eastern U.S.	Ainslee (1927) Noble (1932)
Exorista nigripalpis Towns.	*mutabilis* Clemens *trisectus* Walker	Indiana Indiana	Ainslee (1927) Ainslee (1927)
Zenillia caesar Aldrich	undet. spp.	Eastern U.S.	Noble (1932)
Aplomyia confusionis Sllrs.	*bonifatellus* (Hulst) *sperryellus* Klots	California California	Original records Original records
	Fungi		
Isaria sp.	*mutabilis* Clemens	Tennessee	Ainslee (1923*a*)
Empusa sp.	undet. spp.	Ontario, Canada	Stirret and Arnott (1932)
Beauveria bassiana (Bak.)	undet. spp.	Indiana	Noble (1932)

NATURAL ENEMIES

Lawn moths have numerous natural enemies, whose abundance varies from year to year and whose value in control is difficult to estimate. Although many species appear to be casual predators, a few almost always accompany lawn moths and may assist in limiting their activities and in checking the reinfestation of treated lawns.

PARASITES

So far as can be determined, 15 species of insect parasites and 3 species of fungi have been recorded in the literature as preying upon North American species of *Crambus*. This information is summarized in table 9. Three species of parasites have been reared by the writer from California species. All these are in genera reported as parasites on eastern sod webworms.

Orgilus.—Parasites reared from pupal shelters of *Crambus bonifatellus* and *C. sperryellus* in Los Angeles, and collected by sweeping over turf there and in Redding and Bakersfield, were identified by C. F. W. Muesebeck as belonging to a new species of braconid of the genus *Orgilus* (figs. 12 and 13, *C* and *D*). The species was observed in large numbers flying over Los Angeles lawns in August and September, 1941. Of the *C. bonifatellus* pupae taken from one such lawn on August 8, 1941, 40 per cent had been parasitized by *Orgilus*. Known stages of the species concerned may be briefly described as follows:

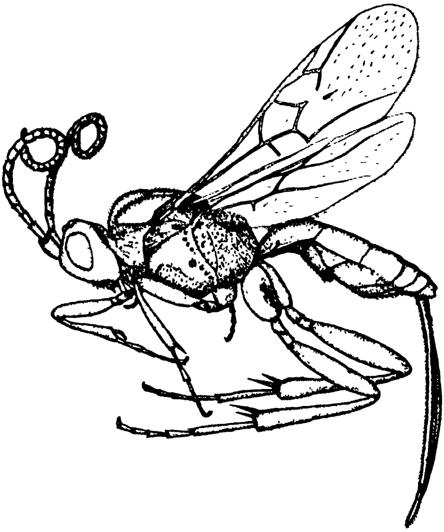

Fig. 12.—*Orgilus* sp., a larval parasite of *crambus bonifatellus* and *C. sperryellus.*

Female: black with red legs, labrum and mandibles partly reddish, abdomen red and black, with the red sometimes restricted mainly to the second tergite; wings dusky, stigma dark brown; length of body (excluding antennae and ovipositor) 3.5 mm; length of antenna about 2.7 mm; length of ovipositor measured from tip of abdomen 2.5 mm. Male: markings as in female; length of body (excluding antennae) 3.5 mm; length of antenna 4.0 mm. Cocoon formed within the pupal shelter of the host; oval, golden brown, translucent, parchmentlike, covered thinly with silk fuzz; length 4.5 mm; greatest diameter 2.0 mm.

Apanteles.—Specimens of parasites reared from prepupal larvae of *Crambus bonifatellus* and *C. sperryellus* infesting a Los Angeles lawn were determined by C. F. W. Muesebeck as a new species of *Apanteles* (fig. 13, *A*). This wasp appears common throughout the Los Angeles area, having been collected for several years on lawns in Inglewood, Southgate, San Gabriel, Pasadena, Hollywood, Van Nuys, West Los Angeles, and Culver City. The known stages are briefly described as follows:

Female: black with yellowish-red legs, which are darkened at the apices of the femora and tibiae; wings nearly transparent; the veins and stigma pale brownish. Length of body (excluding antennae and ovipositor) 2.0 mm; length of antenna 1.7 mm; ovipositor extruding about 0.6 mm beyond end of body. Male: coloration about as in female except that the first 4 abdominal sternites are yellowish red. Length of body (excluding antennae) 2.0 mm; length of antenna 2.0 mm. Cocoon: oval, tightly woven of glistening white silk with stray threads scattered over the surface; opening by a circular hinged cap at the anterior end. Length 3.0 mm; diameter about 1.2 mm.

Aplomyia.—A species of tachinid fly reared from larvae of *Crambus bonifatellus* and *C. sperryellus* collected in Riverside and in Los Angeles was identified by H. J. Reinhard as *Aplomyia confusionis* Sllrs. (fig. 13, *B*). The adults were commonly collected by sweeping infested lawns in Riverside and Los Angeles. The known stages are briefly described as follows:

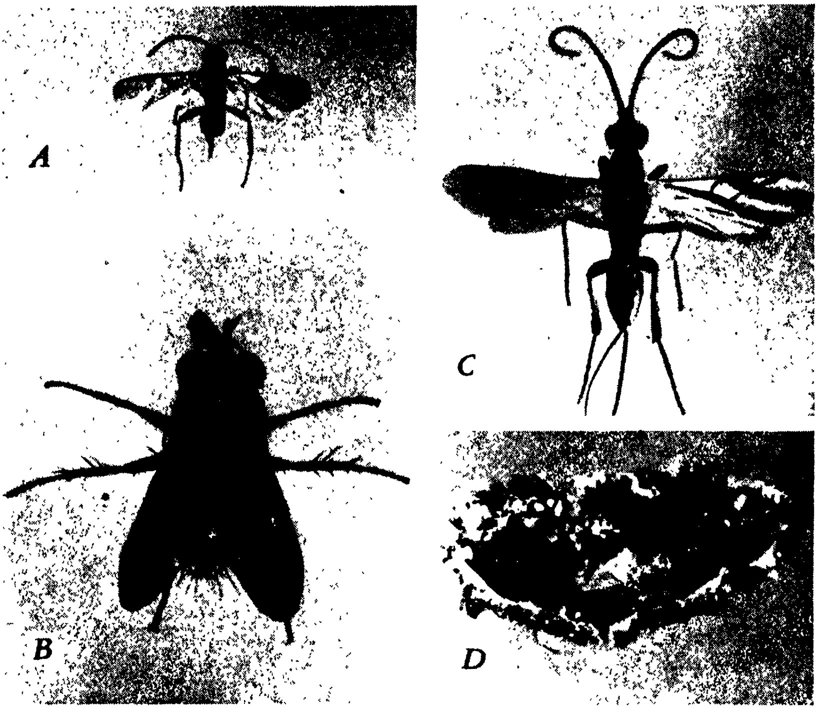

Fig. 13.—Parasites of *Crambus* spp. in California lawns: *A*, *Apanteles* sp.; *B*, *Aplomyia confusionis* Sllrs.; *C*. *Orgilus* sp.; *D*, pupal cocoon of *Crambus bonifatellus* containing cocoon of *Orgilus* sp.

Female: black, with a silvery pollinose covering that is especially prominent on the face and at the bases of the second and fourth abdominal tergites; compound eyes brick red in life; dorsum of thorax with 5 poorly defined, broad, pollinose stripes. Length of body 6.0 mm; breadth of abdomen 2.2 mm. Puparium, formed after emergence from the host pupa: broadly oval, pale brownish red. Length 5.0 mm; greatest breadth 2.5 mm.

DISEASES

A few records have been published of fungus diseases and undetermined bacterial and virus diseases that attack eastern species of *Crambus*. At certain times, notably in late fall, the writer has witnessed sudden decreases in sod-webworm population that apparently could not be attributed to the weather, to predators, or to insect parasites. Larval diseases may occasionally have been responsible. In the laboratory, larvae reared at 75° and 86° F often sickened in the fourth and following instars and died after a paralysis lasting a day or two. The body contents then rapidly decomposed into a dark-brown liquid. Both species of lawn moths were affected, especially *C. sperryellus*. Dead and nearly dead larvae bore no evidence of fungus. Four species of bacteria were isolated from sick larvae by A. J. Salle.[6] These were a small Gram-negative rod (probably *Escherichia coli*), a short and fat Gram-negative rod, a species of *Staphylococcus*, and a species of *Streptococcus*. Broth cultures of these four bacteria were poured over grass, which was then fed to *Crambus* larvae; but no increase in mortality took place as compared with larvae fed upon untreated grass.

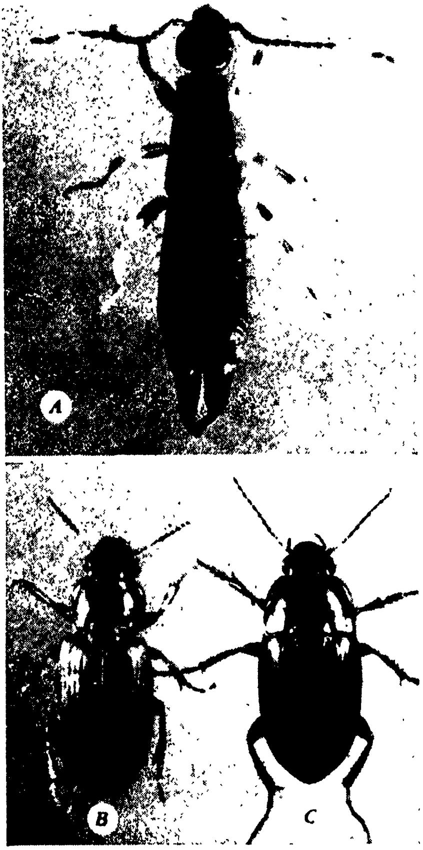

Fig. 14.—Predators on *Crambus* spp. in California lawns: *A*, the ring-legged earwig, *Euborellia annulipes* (Lucas); *B*, *Agonoderus lineola* Fab.; *C*, *Celia californica* (Dej.).

PREDATORS

Birds.—Despite their beneficial activities as predators on insect larvae in the grass, birds are sometimes a nuisance on the putting greens of golf courses, where they tear up the turf in search of food. Most greenkeepers believe, however, that the benefit derived from the birds offsets this damage.

By far the most common insectivorous bird on California lawns is the Brewer blackbird, *Euphagus cyanocephalus* Wagl.[7] The writer has frequently seen many hundreds of these in a single flock searching systematically over a lawn for insects. Several times it was possible to approach closely enough to identify their prey, which was chiefly sod webworms, cutworms, skipper larvae, and angleworms. Other birds observed feeding on lepidopterous larvae in lawns are the meadow lark, *Sturnella neglecta* (Aud.); the killdeer plover, *Aegialitis vocifera* (Linn.); the western mockingbird, *Mimus polyglottos leucopterus* (Vigors); the robin, *Turdus migratorius* Linn.; and the shrikes, *Lanius* spp.

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[6] Professor of Bacteriology, University of California at Los Angeles.

[7] Determinations of birds in this paragraph by Loye H. Miller, Professor of Biology, Emeritus, University of California at Los Angeles.

Vespid Wasps.—The common yellowjacket, *Vespula pennsylvanica* (Saussure), is a robust black and yellow wasp with a short, stout abdomen. The workers average about 11 mm in length. They are occasionally seen about lawns, and in one instance large numbers were observed carrying off sod-webworm larvae from a severe infestation.

Polistes spp. are moderately elongate wasps, with an abdomen that tapers toward the base but has no long, narrow petiole. The workers average about 16 mm in length. Two species are common in California, *P. fuscatus aurifer* Saussure, and *P. anaheimensis* Provancher. The former is black and yellow; the latter chiefly red and yellow. Wasps of both species have been observed several times while carrying off lawn-moth larvae.

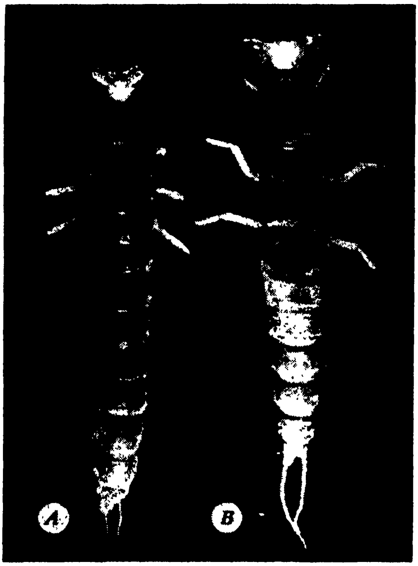

Fig. 15.—Larvae of two common carabids predaceous on *Crambus* spp. in California lawns: *A*, *Agonoderus lineola* Fab.; *B*, *Celia californica* (Dej.).

The Ring-legged Earwig.—*Euborellia annulipes* (Lucas) is a common lawn inhabitant in southern California (fig. 14, *A*). It is a wingless species, about 15 mm long at maturity, reddish brown, the antenna subapically annulated with whitish, and the yellowish leg spotted with brown. The stout forceps can close tightly along the entire inner surface. This species is probably an important insect predator on lawn-infesting caterpillars. In laboratory tests, larvae of *Crambus bonifatellus* were quickly seized and crushed between the powerful forceps of the earwigs. If a larva continued to struggle, the earwig gripped it at one end with the forceps and began feeding on the other end. In an overnight test one female earwig consumed 25 first-instar larvae of *C. bonifatellus,* and another dealt similarly with 25 eggs of the species. According to Klostermeyer (1942), the ring-legged earwig is cosmopolitan in distribution and though omnivorous, prefers an insect diet.

Predaceous Ground Beetles.—Two carabids, *Celia californica* (Dej.) and *Agonoderus lineola* Fab. (fig. 14, *C* and *B*), are frequent lawn inhabitants in southern California. The former is a glossy black beetle 12 to 13 mm long, the antennae reddish basally, the tibiae and tarsi brownish, and the elytra with fine longitudinal striae. The mature larva is about 20 mm long, including the bristly caudal processes, which measure about 4.5 mm (fig. 15, *B*). The larval head and pronotum are reddish brown, the mesonotum, metanotum, and legs brownish yellow; the abdomen is gray. The mandibles are slender and untoothed.

The adult of *Agonoderus lineola* is 10 to 13 mm long and is brownish yellow, marked with brown or black. Two indistinctly margined longitudinal brown lines on each elytron are characteristic. The mature larva is about 20 mm long, including the bristly caudal processes, which measure 2 mm (fig. 15, *A*). The larval head and thorax are brownish yellow, and the abdomen is gray. The stout mandibles have a large tooth on the inner surface.

According to laboratory tests, adults and larvae of both these species of carabids feed readily on larvae and eggs of sod webworms. Considering their abundance, these beetles may often be important predators on *Crambus* in lawns.

Rove Beetles.—Numerous species of staphylinids live in lawns. Many of these are potential predators on sod webworms.

CONTROL OF LAWN MOTHS

Control studies were carried out by the writer from August, 1938, to October, 1942. Since that time a number of new materials, such as DDT, have been introduced and it appears likely that they will have a place in lawn-insect control. As the new insecticides have not yet been evaluated, only a summary of results is presented now.

As reported in the literature (Bohart, 1940; Houser, 1931; Hutson, 1933; Jewett, 1939; North and Thompson, 1933; Price, 1931; Stone and Elmore, 1937), materials most widely used for sod-webworm control were pyrethrum compounds, lead arsenate, and dichloroethyl ether.

Materials tested by the writer were pyrethrum compounds, rotenone compounds, ground tobacco, nicotized powder, cuprous cyanide, dichloroethyl ether, cryolite, and various arsenical compounds. When used in practical quantities, all of these except the ground tobacco and nicotized powder killed a high percentage of larvae present in the grass at the time of application. In some years, however, particularly with an early summer, reinfestation of lawns treated with pyrethrum compounds, rotenone compounds, and dichloroethyl ether was a serious problem, 3 or more applications being required to keep the grass in good condition. The remaining materials had a more lasting effect, but cuprous cyanide was not generally available and was expensive, and cryolite had an unfavorable reaction on the grass.

Arsenicals used were acid and basic lead arsenate, and calcium arsenate. Of these the acid lead arsenate gave best results. One application was generally sufficient to control lawn moths for the season; occasionally a second treatment was necessary.

Acid lead arsenate is better used as a spray than as a dust because of the greater adhesion and more even distribution. If the lawn is not too large, the application can be made with a sprinkling can. Experience has shown that 1 man can spray 1,000 square feet in about an hour. A thorough mixture can be obtained by placing a measured amount of lead arsenate in the bottom of a sprinkling can and filling the can with a strong spray of water from a hose nozzle. The dosage found most satisfactory was 5 pounds of lead arsenate to 50 gallons of water applied to 1,000 square feet of sod. With most brands of lead arsenate this is equivalent to 1¼ standard measuring cups of insecticide (4.8 ounces) to 3 gallons of water applied to 60 square feet. For convenience, 10-foot and 6-foot sticks can be used to mark off unit areas of the lawn as the treatment progresses.

DISEASES AND MINOR PESTS OF CALIFORNIA LAWNS

Many pests and fungus diseases are encountered in lawns. These may act separately or together in damaging the grass; in the latter case, diagnosis is often difficult. The following are notes on certain common pests and diseases observed by the writer in California.

Skippers.—The fiery skipper, *Hylephila phylaeus* Drury (Hesperiidae), is a common lawn inhabitant in California. According to Comstock (1927) it is "an exceedingly abundant butterfly throughout the lowlands of southern California. Its range extends northward to the San Francisco Bay district, southward to Argentina and east to the Atlantic coast." The writer has collected the species in many parts of California.

Despite its widespread abundance, it is only occasionally reported as injuring lawns. Five such instances have been observed, one each in Redding, Riverside, and Montebello, and two in Los Angeles. The lawn involved was always of bent grass. In the early stages the infestations were marked by isolated round spots, 1 to 2 inches in diameter, produced by the feeding of individual larvae. In two of the lawns observed, the spots had coalesced, and most of the lawn was killed. Although injury to bent grass appears to be most severe, larvae will feed on all the common lawn grasses.

In the laboratory, specimens were reared at about 75° F on Bermuda grass in an average of 48 days from egg to adult. The hemispherical eggs are glued singly to grass blades (fig. 16, *D*) as the female lights on the lawn during the heat of the day. The egg has an average breadth of 0.70 mm. The larva is about 25 mm long and has a unique, strongly constricted neck (fig. 16, *C*). The black head is mottled or striped with brown, and has a granular surface. The body is brownish yellow, sometimes with indistinct longitudinal stripes. The pronotal shield is a narrow transverse black band, and the caudal plate a mottled brown. The pupa, 15 to 18 mm long, is rather thickly covered with bristly hairs. It is yellowish brown, with a conspicuous black dorsal line that is broken toward the end of the abdomen, and with two prominent black dashes on the thorax parallel to the median line. The pupa is found free in the grass-root zone near the surface. The adult has a wingspread of 23 to 30 mm. The male has orange-yellow wings spotted with black, whereas the female is dark brown with orange-yellow spots (fig. 16, *A*).

Conspicuous white cottony masses in the lawn indicate the presence of skippers. These masses are the pupal cocoons of a braconid, *Apanteles* sp. (determined by C. F. Muesebeck). This parasite (fig. 16, *B*) attacks the larvae of *Hylephila phylaeus* and may constitute an effective check on the species. Another parasite, *Amblyteles* n. sp. (determined by R. A. Cushman), has been reared from pupae of the fiery skipper collected in Santa Rosa, Los Angeles, Montebello, and Riverside.

From observations made on lawns treated for sod webworms, *Hylephila phylaeus* larvae appear somewhat less susceptible to pyrethrum, dichloroethyl ether, and acid lead arsenate sprays than the *Crambus* species. In two tests supervised by the writer, rather satisfactory control on heavily infested bent-

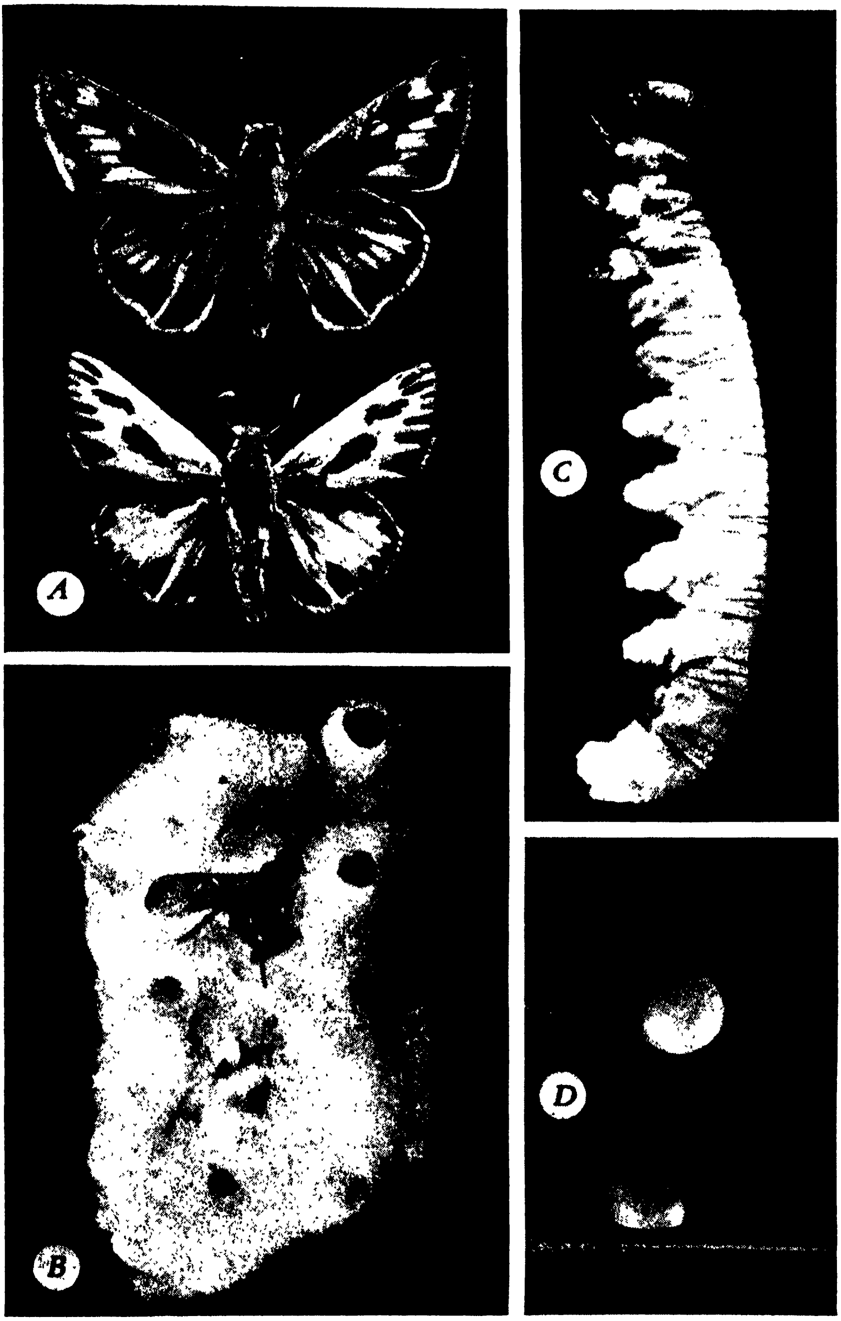

Fig. 16.—The fiery skipper, *Hylephila phylaeus* Drury, and its principal parasite: *A*, adult female (upper) and male (lower) of *H. phylaeus*; *C*, the mature larva; *D*, eggs in natural position on grass blades (greatly magnified); *B*, adult and cocoon mass of *Apanteles* sp. near *A. hesperidivorus* Vier., reared from a fiery skipper larva.

grass lawns followed an application of acid lead arsenate spray, 5 pounds in 50 gallons of water to 1,000 square feet. For best results, probably, the lead arsenate should be increased to 10 pounds.

Cutworms.—Three species of cutworms are commonly found in lawns (fig. 17). Occasionally they may cause severe injury, particularly to bent grass. In the lawn their presence is usually indicated by small spots, 1 or 2 inches in diameter. Close examination of such a spot should reveal definite evidence of feeding, with the grass chewed below the mowing level, and with a hole about the thickness of a lead pencil leading to the grass roots where the cutworm is lodged.

The armyworm, *Cirphis unipuncta* (Haw.), is about 40 mm long at larval maturity. The head is yellow, mottled with brown; and the dark-gray body is ornamented with 5 longitudinal yellow stripes, 3 on the dorsum and 1 on each side. The pupa is about 15 mm long and dark reddish brown. The adult has a wingspread of 37 to 42 mm (fig. 17, *A*). The forewing is tan, with a small silvery eyespot that subtends an oblique dark line reaching the tip of the wing. The hind wings are light on the basal half, dark on the apical half, and have dark venation. In the laboratory, specimens reared at 75° F on bluegrass took an average of 48 days from egg to adult.

The granulate cutworm, *Feltia subterranea* (Fab.), is about 35 mm long at larval maturity. The head is mainly dark brown, but variegated with lighter brown. The body, dark gray mottled with black, presents a granulated appearance. The adult's wingspread is 28 to 38 mm (fig. 17, *C*). The forewing has a light tan ground color and black markings. Two pale eyelike marks separated by a black bar appear in the upper middle of the wing, and a large subapical black spot is present along the anterior wing margin. The hind wings are silvery white, with pale venation. A single specimen of *F. annexa* was reared to maturity at about 75° F on bluegrass cuttings. The egg, larval, and pupal stages took 5, 24, and 9 days, respectively, or a total from egg to adult of 38 days.

The variegated cutworm, *Peridroma margaritosa* (Haw.), is about 45 mm long at larval maturity. It is gray or brown with blotchy gray and black lateral markings, and with a median dorsal row of pale, small, somewhat diamond-shaped spots. The adult has a wingspread of 37 to 47 mm (fig. 17, *B*). The forewing is a mottled dark gray or brownish gray, often with a purplish tint. In the upper middle of the wing are two indistinct eyelike spots. The hind wings are whitish to gray, with dark venation.

According to commercial operators, a poisoned bran bait for cutworms in lawns has not proved satisfactory. An infestation can probably be controlled by applying 10 pounds of acid lead arsenate in 50 gallons of water to 1,000 square feet. In two instances observed by the writer, this treatment appeared successful.

Pyralids.—The lucerne moth, *Nomophila noctuella* (D. and S.) (fig. 17, *D*, and fig. 18), occurs in lawns throughout California. As the common name implies, it is primarily a pest of alfalfa. It likes other legumes, including white clover, but in their absence will feed on grass. In laboratory rearings the larvae were fed various lawn constituents; they seemed definitely to prefer white clover to bluegrass, orchard grass, or Bermuda grass. Specimens reared on white clover at about 75° F took an average of 32 days from egg to adult. In Illinois, according to Flint (1922), the species damages red clover, sweet clover, and alfalfa, but also feeds upon corn, purslane, bluegrass, white clover, mustard, cinquefoil, foxtail, and soybean.

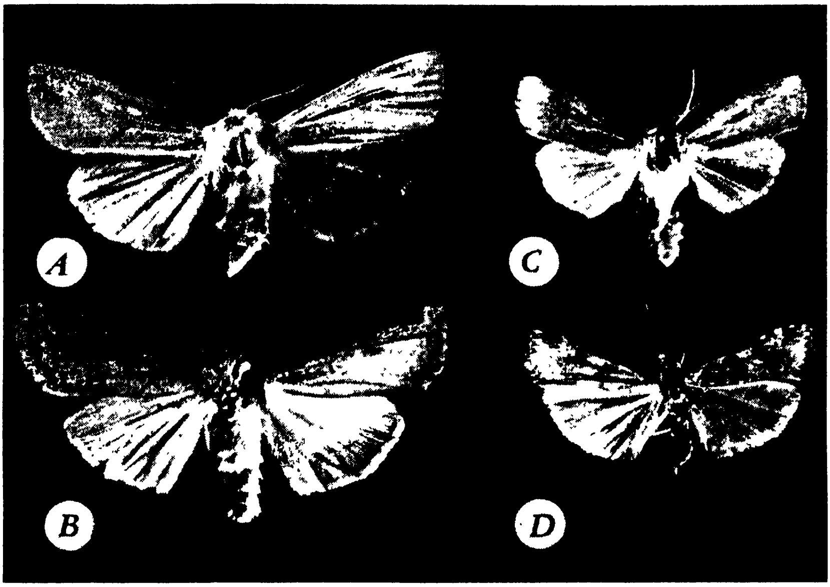

Fig. 17.—Common lawn-infesting moths: *A*, the armyworm, *Cirphus unipuncta* (Haw.); *B*, the variegated cutworm, *Peridroma margaritosa* (Haw.); *C*, the granulate cutworm, *Feltia subterranea* (F.); *D*, the lucerne moth, *Nomophila noctuella* (D. and S.).

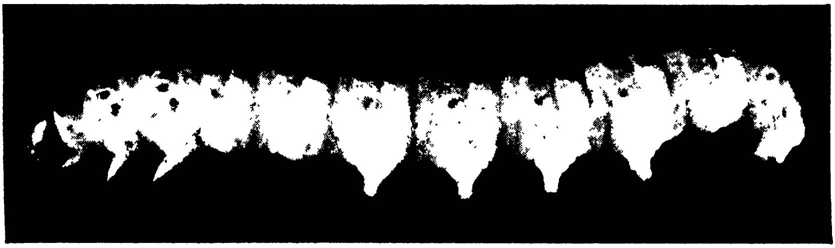

Fig. 18.—Mature larva of the lucerne moth, *Nomophila noctuella* (D. and S.).

Adult females collected at lights deposited clumps or rows of 5 to 50 eggs at a time on the host plants. The mature larva is slender, very active, and about 25 mm long, with a black or dark-brown head. The dorsum of the body is ornamented with conspicuous subspherical dark spots; these distinguish the larva from *Crambus bonifatellus,* in which the spots are much less uniformly shaped. The pale-brown pupa is formed within a translucent silken cocoon in the ground. The adult has a wingspread of 25 to 30 mm; and its forewing is a mottled gray brown, with two pairs of indistinct dark spots. The hind wings are gray with dark venation.

In the writer's experience, *Nomophila noctuella* has never been more than a contributing nuisance in unhealthy lawns. Apparently it is well controlled by the arsenate spray recommended for sod webworms.

Ommatopteryx texana Rob. has been seen about lawns in southern California from Bakersfield to San Diego. Adults collected at lights laid numerous nearly oval eggs on grass blades with which they were caged. The eggs were loosely attached and were easily dislodged by disturbing the grass blades. The larvae were offered cuttings of white clover, Bermuda grass, and bluegrass, but fed only on the clover. Laboratory specimens reared at about 75° F averaged 57 days from egg to adult. The mature larva is about 17 mm long. The head is almost unicolorous brown; and the body is grayish yellow, with indistinct gray spots. The pupa is formed in a fairly opaque cocoon in the ground. The adult has a wingspread of 18 to 22 mm. The grayish forewing has two oblique transverse yellow lines at about the middle, and a marginal row of black spots edged with gold. The hind wings are whitish to gray, with gray venation.

The California Harvester Ant.—In sandy areas of southern California the California harvester ant, *Pogonomyrmex californicus* (Buckley), often makes itself a nuisance by building nests in lawns. A colony creates a bare spot by clearing away the grass around its nest. The workers can bite and sting viciously. Although calcium cyanide dust poured into the nests and covered with earth is commonly used for control, the colonies are seldom exterminated in this way and will usually reappear. The writer, in a limited number of tests, employed the following methods: (1) Calcium arsenate dust, both alone and mixed with flour, was forced into the nests under pressure. (2) Cuprous cyanide dust was forced into the nests under pressure. (3) Dichloroethyl ether in concentrated form and in a 1 to 400 dilution in water was poured into nests. (4) A pyrethrum product[8] in concentrated form and in a dilution of 1 to 400 in water was poured in. Each of these treatments reduced the numbers of ants and often caused the colony to move its entrance a short distance away. Apparent extermination was achieved with the diluted dichloroethyl ether and the diluted pyrethrum product if 2 gallons or more were poured into the nest.

The California Fire Ant.—*Solenopsis xyloni maniosa* Wheeler is a small reddish and black ant, known as the California fire ant because of its vicious sting. Colonies occasionally occur in lawns, creating large spongy areas around their nests. The pest may seriously injure the grass by chewing on the roots and crown. An apparently effective control is described by Mallis (1938): A mixture of 1 part carbon disulfide and 3 parts carbon tetrachloride is poured into holes made with a screwdriver in the nesting site. The holes are then covered with earth and with wet burlap sacks to confine the fumes. For satisfactory results this treatment may have to be repeated two or three times over a period of 2 weeks.

The Argentine Ant.—The common house ant or Argentine ant, *Iridomyrmex humilis* Mayr, is a frequent and unwelcome visitor in lawns. Usually it can be controlled by a persistent use of commercial arsenical ant sirups.

White Grubs.—The larvae of Scarabaeidae, known as white grubs, often feed upon grass roots. When well grown they are fleshy whitish grubs ½ to 1 inch long and partially coiled, like the letter C. Their damage to California lawns is apparently sporadic, and little injury has been reported during the past few years. The most abundant lawn-infesting species of this group in southern California is *Spilosota hirta* (Lec.). The adult beetle is about 15 mm long, mostly yellowish brown, with a reddish head, black eyes, and two indistinctly outlined longitudinal brown lines on each elytron (fig. 19, *C*). In Los Angeles, Riverside, and intervening cities, the beetles are attracted to lights on warm midsummer evenings. Each year there is apparently a concentrated flight of adults, lasting about 2 weeks. Before and after this period the beetles are rarely encountered. The writer observed heavy flights in West Los Angeles on July 6, 1939, July 1, 1940, and June 23, 1941. In 1941, regular weekly observations were made there from February until November. The first adult *Spilosota hirta* was observed on June 17, and the last one on July 1. Also in 1941, flights of beetles were seen in Riverside on June 14 and in Pasadena on June 19. During all these flights, certain characteristics were observed: (1) The beetles appear at dusk in large numbers (about 1 to the square yard at the peak) and mostly disappear in about 20 minutes. (2) They fly over the lawn in zigzag fashion at a height of 1 or 2 feet. (3) During and shortly after these flights a few beetles, representing a very small proportion of the population, are attracted to street lights.

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[8] Containing 2 per cent pyrethrins.

In a severe infestation of white grubs, practically all the grass roots are severed, and the lawn can be easily rolled back to expose the larvae at work.

Control methods have been carefully worked out for eastern species of lawn-infesting white grubs and should also be successful under California conditions. Hadley (1940) made the following statements concerning the treatment of lawns and other turf areas for white grubs of the Japanese beetle:

> An application of lead arsenate at the rate of 10 pounds to 1,000 square feet of turf area is recommended. This small quantity of lead arsenate must be diluted to a greater bulk so that it can be uniformly distributed over the surface. The material may be mixed with about 25 times its volume of moist sand, soil, or other suitable material, and broadcast by hand, or with a hand-operated fertilizer distributor. Immediately after lead arsenate has been applied the lawn should be well watered with a hose to wash the poison into the soil. It may be expected that a lawn treated in this manner will be immune to injury by the grubs for at least 5 years.

Billbugs.—The larvae of several species of the genus *Calendra* (Curculionidae) frequently occur in lawns. They are small, white, legless grubs approximating ¼ to ⅜ inch in length at maturity. Van Dyke (1938) recorded *C. minimus* (Hart), *C. reticulatus* (Boh.); *C. sequoiae* V. D., *C. simplex* (Lec.), and *C. gentilis* (Lec.) from lawns in the San Francisco Bay area. Species collected in turf by the writer are *C. phoeniciensis* (Chittn.) (fig. 19, *A*), *C. tarda* (Fall), *C. glyceriae* (Chittn.), and *C. sequoiae* V. D. (all four from southern California). Two severe infestations of *C. tarda* in bent grass have come to the writer's attention. One, in San Gabriel, was treated with three sprays, dichloroethyl ether (1 part to 200 parts of water and 1 gallon to the square yard), acid lead arsenate (10 pounds in 50 gallons of water to 1,000 square feet), and calcium arsenate[9] (10 pounds in 50 gallons of water to 1,000 square feet). According to a survey 2 weeks after treatment, dichloroethyl ether had given an excellent kill. Many dead larvae and no live specimens were found near the grass roots. The two arsenicals, however, gave only about a 50 per cent control as compared with the check. A similar infestation involving *C. phoeniciensis* was observed on Bermuda grass at Alhambra, in September, 1942. At that time much of the lawn had been killed, and crabgrass had begun to fill in the earlier-killed patches. Both adults and larvae of the billbugs abounded in the partially green grass on the fringes of dead areas. According to the gardener, lead arsenate sprayed several weeks before had failed to give satisfactory control.

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[9] Containing 30 per cent inert ingredients, including other calcium compounds.

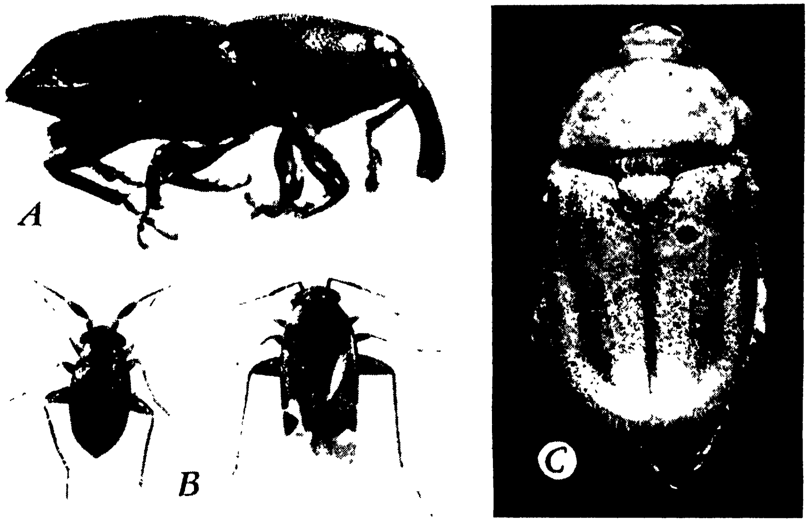

Fig. 19.—Grass-infesting insects: *A, Calendra phoeniciensis* (Chittn.); *B,* nymph and adult of *Leucopoecila albofasciata* Reut.; *C, Spilosota hirta* Lec.

Mirids.—A common gray and white saltatory bug found in California lawns is *Leucopoecila albofasciata* Reuter (fig. 19, *B*). If it becomes numerous, the infested lawns show retarded growth or may die out in spots. Bluegrass, Bermuda grass, and bent grass appear to be most affected. This pest is widely distributed over the United States and, according to Knight (1941), has damaged golf greens in Missouri and New York. Hamilton (1935) recommended a derris dust, a nicotine and pyrethrum dust, a nicotine sulfate and soap spray, or a pyrethrum and soap spray for controlling it on New Jersey lawns and golf courses. In a single test made by the writer, good results were obtained with a pyrethrum spray (containing 2 per cent pyrethrins) diluted 1 to 400 with water, 2 gallons being applied to 100 square feet with a 3-gallon hand-pump sprayer; also with a pyrethrum dust (0.1 per cent pyrethrins), 2 pounds to 1,000 square feet. The materials acted rapidly; most of the bugs in the treated areas were apparently dead after 10 minutes. A pronounced recovery of the grass was noted 2 weeks later.

Leafhoppers.—Many species of leafhoppers (Cicadellidae) live in lawns. Ordinarily the effect of their feeding is not noticeable if the grass is well watered and regularly fertilized. In California the species most frequently injurious appears to be *Draeculacephala mollipes* (Say). Osborn (1939) gives the life history and habits of this widely distributed green and yellow leafhopper. According to reports of commercial operators, it is easily controlled with pyrethrum in either spray or dust form.

Mites.—A grass mite, *Paratetranychus stickneyi* McGregor, common on Bermuda grass in southern California, can be considered a potential pest. Apparently this mite thrives when the grass is in poor condition through lack of moisture.

Pillbugs and Sowbugs.—The common pillbug, *Armadillidium vulgare* (Latr.), and the dooryard sowbug, *Porcellio laevis* Koch, are frequent lawn inhabitants (fig. 20). Although these crustaceans feed principally on dead and decaying grass, they constitute a nuisance; and a lawn is often a focal point from which other plants are attacked. Their control has been discussed by Bohart and Mallis (1942). Arsenical poison baits containing bran or vegetable meals are ordinarily recommended for sowbug and pillbug control, but appear not to be satisfactory on lawns. During experiments with sod webworms, acid lead arsenate at 10 pounds to 1,000 square feet and calcium arsenate[10] at 3 pounds to 1,000 square feet killed a high percentage of pillbugs.

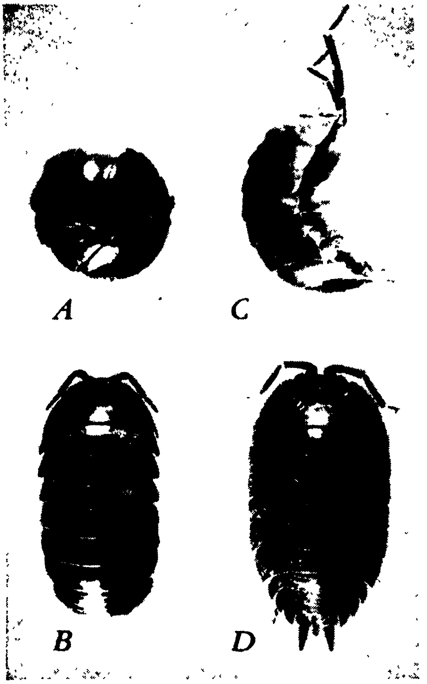

Fig. 20.—*A*, *B*, The common pillbug, *Armadillidium vulgare* (Latr.); *C*, *D*, the dooryard sowbug, *Porcellio laevis* Koch.

Fungus Diseases.—Eleven major lawn diseases have been discussed by Monteith and Dahl (1932): brown patch (*Rhizoctonia solani*), dollarspot (*Rhizoctonia* sp.), spotblight (*Pythium butleri*), snowmold (*Fusarium nivali*), damping-off (various fungi), leafspot (*Helminthosporium vagans*), zonate eyespot (*Helminthosporium giganteum*), fairy ring (various fungi), smut (*Ustilago striaeformis*), rust (*Puccinia* spp.), mildew (*Erysiphe graminis*). Of these, by far the most common in California, and the most apt to be confused with lawn-moth damage, are brown patch and dollarspot.

According to the authors just cited, brown patch occurs in more or less circular spots, varying from 1 inch to 3 feet or more in diameter. The grass in the center of the affected area turns first dark and then, gradually, a light brown. On the outer borders there is usually a dark ring of more recently affected grass, which indicates that the fungus is still actively spreading. In light attacks not all the grass blades are killed, and the spot may retain a light greenish color. The disease affects various lawn constituents, including bent grass, Kentucky bluegrass, ryegrass, Bermuda grass, and clover. Two types of bent grass, colonial and redtop, are particularly susceptible.

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[10] Containing 30 per cent inert ingredients, including other calcium compounds.

Dollarspot, a similar disease, usually appears in patches less than 2 inches in diameter, gives the dead grass a bleached straw color, and thrives at a somewhat lower temperature and in a more acid soil than the brown patch. Though creeping bent is most susceptible, dollarspot attacks many other lawn constituents. Colonial bent, redtop, and especially velvet bent are resistant. Both diseases are favored by excessive rainfall, heavy dews, fog, cloudy weather, high humidity, and high temperatures. One can distinguish them from sodwebworm injury by examining the individual grass blades. In fungus infections the brown spots are produced by the dead grass, whereas in caterpillar damage the blades are partially or wholly removed, and the brown color results mainly from the short stubble and from the exposed ground. In doubtful cases one should apply a pyrethrum solution to test for the presence of insects (see footnote 5, p. 270.

In the opinion of Monteith and Dahl (1932), brown patch and dollarspot are best controlled by mercury compounds. Fungicide manufacturers are now, however, offering organic sulfur substitutes.

SUMMARY

The two chief lawn-infesting insects in California are *Crambus bonifatellus* (Hulst) and *C. sperryellus* Klots. These, the only representatives of the genus *Crambus* known to attack lawns in California, are easily distinguished by adult, larval, and pupal characters. In the adult, the forewings of *C. bonifatellus* are brown, variegated with buff, whitish, and black; whereas those of *C. sperryellus* are golden, with a longitudinal silver stripe. Young larvae of the two species are similar in appearance; but after the third molt, the head of *C. sperryellus* has much more distinct spots than that of *C. bonifatellus*. The pupa differs especially in the caudal process, which is blunt in *C. bonifatellus* and roundly pointed in *C. sperryellus*.

On the basis of the wing patterns, the adult *Crambus bonifatellus* is called the fawn-colored lawn moth whereas *C. sperryellus* is designated as the silver-barred lawn moth.

Many species of the genus *Crambus* in various parts of the world have been recorded as injuring agricultural crops, especially those of the grass family. Their importance as pests of lawns and golf courses in North America was not generally realized until the widespread drought of 1929 to 1932. The first report of injury to lawn grass in California was published in 1929, and since then these larvae (sod webworms) have taken a large yearly toll.

Crambus bonifatellus occurs throughout the Canadian zone of the entire Rocky Mountain and Great Basin area. In the Northwest it has been collected in British Columbia and Oregon. In California it occurs near the sea—rarely inland. *C. sperryellus* is restricted mainly to California, where it is common along the coast and throughout the Sacramento and San Joaquin valleys. In southern California it ranges inland as far as the San Bernardino Mountains, Needles, and the Coachella Valley.

In the field, lawn moths apparently prefer bent and young bluegrass lawns. In laboratory feeding tests, many other grasses and clover were found to be potential host plants.

The most reliable test for sod webworms is to apply a pyrethrum-containing solution to various parts of a lawn in order to bring the larvae wriggling to the surface. Other criteria of infestation that can be used are irregular brown patches near which the grass is thin and uneven, and an abundance of parasites and predators in or hovering about the turf.

Eggs are scattered by the female moth during the oviposition flight, which begins just before dusk and lasts about an hour. A few moths are attracted to artificial lights toward the end of the oviposition period and sometimes for several hours thereafter. According to laboratory tests an average female lays 200 to 300 eggs, with exceptional individuals producing over 500. *Crambus bonifatellus* appears to be somewhat more fecund than *C. sperryellus*.

In the laboratory *Crambus bonifatellus* was reared from egg to adult in an average of 35.9 days at about 75° F. Of this period 4.5 days were taken by the egg, 23.7 days by the larva, and 7.7 days by the pupa. At 59° the average time from egg to adult was 133.7 days. Six larval instars are the rule at about 75°.

Crambus sperryellus was reared from egg to adult in an average of 54.8 days at about 75° F in the laboratory. Of this period 6.0 days were taken by the egg, 37.6 days by the larva, and 11.2 days by the pupa. Seven larval instars are the rule at this temperature.

The California sod webworms infesting lawns appear to breed continuously throughout the late spring, summer, and early fall. The period from late October to late April is customarily passed in a semiquiescent state by mature or nearly mature larvae. In the Los Angeles area *Crambus bonifatellus* apparently completes four generations a year, and *C. sperryellus* only three.

Three species of parasites have been reared from California sod webworms infesting lawns—two braconids, *Orgilus* sp. and *Apanteles* sp.; and a tachinid, *Aplomyia confusionis* Sllrs. Occasionally these appear to play a major part in control. The more important predators are the Brewer blackbird, vespid wasps, a native species of earwig, carabids, and rove beetles.

Several insecticides when applied in sufficient concentration gave adequate temporary control of sod-webworm infestations—namely, pyrethrum and rotenone products, dichloroethyl ether, and acid lead arsenate. Of these only the lead arsenate prevented reinfestation. The most satisfactory treatment is 5 pounds of acid lead arsenate in 50 gallons of water, applied to 1,000 square feet.

The chief pests other than sod webworms in California lawns are the fiery skipper, cutworms, the lucerne moth, the California harvester ant, the California fire ant, white grubs, billbugs, *Leucopoecila albofasciata,* leafhoppers, sowbugs, and pillbugs.

The chief lawn diseases in California are brown patch and dollarspot. These are sometimes confused with injury from sod webworms, but the latter is distinguished by unevenness of grass height and irregularity in the shape of the dead spots. Pyrethrum-containing solution may be used as a conclusive test for the presence of the insects.

ACKNOWLEDGMENTS

The author is grateful to the many persons who have aided him in this study. R. H. Smith, who suggested the problem in 1938, contributed advice throughout, while R. J. Pence assisted with field tests and laboratory studies. Much credit also belongs to C. F. W. Muesebeck and his associates at the U.S. National Museum; to P. C. Ting, H. H. Kiefer, and Loye Miller for identifying specimens; to A. J. Salle for bacteriological investigations; to A. Mallis for advice and help in the work with ants and pillbugs; and to S. F. Bailey for contributing specimens and observations on lawn insects.

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H I L G A R D I A

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DISTRIBUTION OF THE NATIVE GRASSES OF CALIFORNIA[1]

ALAN A. BEETLE[2]

THE GRASSES, supplemented by certain legumes, form the principal basis for range wealth. The natural forage value of the Gramineae as a whole makes an intensive study of their characteristics important, for the broader the knowledge concerning them the more readily may any problem be met. The following paper presents a picture of the current distributions of grasses in California, together with evidences of their floral origins by migration from other regions.

Vegetation has many characteristics which are not always apparent at first glance. For instance, certain elements of the vegetation are native in their location, some are native elsewhere and have only recently been introduced. Some are old species often representative of a primitive condition in their genus, still others appear to be recently evolved. Some of the migrants arrived in California from the north during glacial periods, some crossed the ocean, and others came from the south during interglacial periods. Some plants are distributionally restricted for a number of reasons, including: (1) specialization as to habitat or environmental repression, as the species of vernal pools; (2) recent origin (plants sometimes referred to as neoendemics or initiates), as the endemic varieties of *Distichlis spicata;* (3) ancient origin (paleoendemics or relics); and (4) genotypic specialization (genetic endemics). The limiting factors controlling distribution of grasses are then not always easily recognized, but some attempt has been made to appraise these historical and ecological factors which contribute to an understanding of the present botanical composition of the California rangeland.

Introduced grasses are frequently mentioned in order to complete the overall picture or because of contrast in distributional behavior. Principal attention is paid, however, to the native species, the limits of whose areas are stabilized by climatic and edaphic features interacting over a long period of time. Distributions of 184 California grasses are shown in the maps on pages 319–339. Not all the native species have been mapped but those omitted would not contribute anything new or disturbing. A few introduced species have been mapped where their area of adaptability seemed limited and already occupied.

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[1] Received for publication June 20, 1946.

[2] Assistant Professor of Agronomy and Assistant Agronomist in the Experiment Station; resigned September 5, 1946.

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The first comprehensive list of the grasses of California was made by Bolander (1864–65).[3] At that time he knew 112 species of grasses growing without cultivation, of which 31 were introductions. Soon thereafter Thurber (1880) listed 175 graminaceous species for the state. Now some 400 grasses are known, about one fourth of which are introductions. For convenience in handling the material presented in this study, California has been divided into areas on the basis of recurrent distributions. Neither these areas nor the habitats into which they may be subdivided are wholly contiguous in all their parts. Complex in the same way as biological units, distribution patterns are recognizable but not always disjunct. There are exceptions and overlappings.

THE NORTHERN FLORAL UNITS IN CALIFORNIA

The northern floral units in California occupy the mountain ranges of northern California southward in the Coast Ranges to Monterey Bay and southward in the Sierra Nevada to Tulare County; the mountain peaks of southern California, particularly the San Bernardino and San Jacinto mountains. This delimitation is arrived at after studying the distributions of the native grasses of northern California. Although not all grasses discussed under this heading occupy this whole area they all fall within its limits and are not found outside it. This area is by far the richest in California in numbers of Gramineae. The grasses are consistently of such typically north-temperate genera as *Melica*, *Festuca*, *Poa*, and *Glyceria*. They have all come to California from the north. One half or better are of the tribe Festuceae, about one fourth are of the Agrostideae, one eighth are of the Aveneae, and the remaining one eighth represent all other tribes.

The inclusion of northern California and the coastal pine forests with the Sierra Nevada as a homogeneous floral unit is substantiated by Cooke (1940, 1941) who, in two lists containing 487 species, varieties, subspecies, and forms, could find "no endemic plants"; most of the plants were "fairly common" though this uniform picture may have been disturbed partially by Swallen's subsequent description (1941) of *Glyceria cookei* (map 89). The endemic nature of *Panicum shastense* (map 93) is open to question and depends upon the interpretation of specific limits in *Panicum*.

The pine woods offer the only relief for the agrostologist from the host of introductions that have so altered the aspect of the California flora. Here, alone, native grasses are encountered to the near exclusion of all aliens. Their history is undoubtedly connected with that of the coniferous forests. If the fossil records of these conifers could be coördinated with the known ranges of the grass species and their nearest relatives, considerable light should be shed on the origin of many California grasses.

Northern Grasses That Reach Their Southern Limit in California (maps 1 to 27).—The general predominance of species of or from northern floras that are characteristic of northern California is emphasized when one picks up a flora of Nova Scotia (Dore and Roland, 1941–42) or of Alaska (Scribner and Merrill, 1910) and finds listed in either not only genera but species typical of the northern California floral unit—for example, *Agro-*

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[3] See "Bibliography" for complete citations, referred to in the text by author and date of publication.

pyron trachycaulum, Alopecurus aequalis (map 60), *Cinna latifolia* (map 36), *Calamagrostis canadensis* (map 42), *Deschampsia caespitosa* (map 2), *Koeleria cristata* (map 58), *Trisetum spicatum* (map 35), *Poa palustris,* and *Festuca ovina* (map 55). Of these species, three—*Trisetum spicatum, Deschampsia caespitosa,* and *Koeleria cristata*—reappear in South America, as do *Phleum alpinum* (map 5), *Deschampsia danthonioides* (map 66), *D. elongata* (map 67), and a near relative of *Melica subulata* (map 9).

Of similar wide distribution northward are *Poa pratensis* (map 1), *Agrostis hiemalis* (map 3), *A. exarata* (map 17), *Glyceria pauciflora* (map 7), *G. borealis* (map 11), *G. striata* (map 22), *Festuca rubra* (map 8), *F. subulata* (map 15), *Beckmannia syzigachne* (map 13), *Puccinellia distans* (map 23), and *Deschampsia atropurpurea* (map 26). These grasses are never found in the Great Valley. They are, however, of wide distribution within the limits defined above—that is, they cover a considerable altitudinal range, often occurring in both the Coast Ranges and the Sierra Nevada. In the Coast Ranges they are not found south of Monterey Bay and in southern Sierra Nevada they are not found between Tulare County and the San Bernardino Mountains.

Of somewhat narrower distribution northward but of similar occurrence in California are *Glyceria elata* (map 4), *Trisetum cernuum* var. *canescens* (map 6), *Poa cusickii* (map 10), *Festuca idahoensis* (map 14), *Melica spectabilis* (map 19), *Festuca occidentalis* (map 21), *Puccinellia lemmoni* (map 25), and *Calamagrostis rubescens* (map 27).

Even more restricted but still within the same pattern are *Melica fugax* (map 12), *Festuca subuliflora* (map 20), and *Scribneria bolanderi* (map 24).

The continuous chain of the Sierran peaks is finally broken in Tulare County. A considerable element of the grass flora has, however, been able to bridge the barrier presented by the intervening low elevations and reappears on the San Jacinto, San Bernardino, and other peaks of southern California. The majority of these are found in the San Bernardinos; fewer are known from the other peaks. Species exemplifying this hiatus include, in part:

Agrostis exarata (map 17)
Phleum alpinum (map 5)
Agrostis idahoensis (map 39)
Deschampsia caespitosa (map 2)
Muhlenbergia filiformis (map 37)
Poa bolanderi (map 38)
Muhlenbergia richardsonis (map 40)
Festuca ovina (map 55)
Muhlenbergia andina (map 46)
Festuca viridula (map 48)
Glyceria elata (map 4)

Here they join a small group of grasses including *Muhlenbergia californica* (map 108), *Poa atropurpurea,* and *P. longiligula* (known also from Rock Creek Lake Basin, Inyo County), which are isolated in range but related to the Sierran species. They have not, on the other hand, been able to bridge the gap between these mountains and those of Mexico. True, *Agrostis exarata* is found in Mexico, but its migrational path was not south in the California Sierra Nevada but rather southward in the Rocky Mountain chain through New Mexico and western Texas.

Few annuals are really characteristic of this zone, and these usually occur only at the lower elevations; they are far outranked by the large number of perennials. Some of the introduced annuals listed under open range and savanna, because of their wide tolerance and ready adaptability, are easily found here, but so are *Aira praecox* and *Festuca myuros,* which occur only within the area defined. The natives include *Festuca microstachys, Muhlenbergia filiformis,* and *Poa bolanderi. Muhlenbergia filiformis,* usually listed as a perennial, is treated here as an annual (or at least a very short-lived perennial) because it occurs on sandy stream banks and open washes associated with other very characteristic and typically tiny, annual species including *Eleocharis bella, Cyperus aristatus,* and many others.

The mountain meadows contain an impressive list of economically important introduced perennials:

Agropyron cristatum
Agropyron repens
Agrostis alba
Anthoxanthum odoratum
Arrhenatherum elatius
Bromus inermis
Dactylis glomerata
Festuca elatior
Phleum pratense
Poa bulbosa
Poa compressa
Poa pratensis

Except for sweet vernal grass (*Anthoxanthum odoratum*), a filler usually planted only for the fragrance it gives to hay, all the above introductions are of considerable economic importance. There can be no doubt that meadows in which they are well established are a greater economic resource to their owners than are mountain meadows, usually dominated by *Carex,* where the native vegetation is undisturbed. It must be remembered, however, in attempting to establish these grasses, to which areas of the state they are adaptable. It is not at all surprising that Sampson (1944) had no success in his attempts to reseed burned areas in the chaparral belt with six of the above listed species.

Agrostis alba and *Poa pratensis* have long been known in commercial seed mixtures. They were first brought into cultivation in the Old World and were among the first plants sown for hay in this country by settlers, the origin of the seed being the markets of the Old World. It has then usually been assumed that these grasses were introduced in all their occurrence in this country. Present-day study of ecotypes suggests that these species may have had a panboreal distribution and may have arrived in North America long before the white man. Subsequent crossing of the introduced with the native strains would have complicated the study of the origin of these grasses.

Grasses of High Elevations in the Sierra Nevada (maps 28 to 54).—Many grasses which are common at high elevations (about 7,000 to 9,000 feet) in the Sierra Nevada are not found elsewhere in California, except in the northern Inner Coast Range. In the Sierra Nevada they follow the mountains to Tulare County and then frequently reappear in the San Bernardino Mountains. They are not known in the Great Valley. A few that fit this California pattern are of a very widespread distribution northward—for example, *Trisetum spicatum* (map 35), *Cinna latifolia* (map 36), *Calamagrostis canadensis* (map 42), *Bromus ciliatus* (map 47), and *Danthonia intermedia* (map 51). Most of these grasses are more characteristic of a more restricted area—from British Columbia to Montana southward to California and New Mexico—for example, *Agrostis thurberiana* (map 28), *A. idahoensis* (map 39), *A. rossae* (map 43), *Bromus suksdorfii* (map 45), *Melica stricta* (map 29), *M. bulbosa* (map 44), *M. aristata* (map 30), *Poa pringlei* (map 31), *P. epilis* (map 32), *P. bolanderi* (map 38), *Glyceria erecta* (map 33), *Danthonia unispicata* (map 34), *Muhlenbergia filiformis* (map 37), *M. andina* (map 46), *M. montana* (map 53), *Festuca viridula* (map 48), *Trisetum wolfii* (map 50), and *Stipa webberi.* Only a few are endemic to the Sierra Nevada—for example, *Trisetum cernuum* var. *projectum* (map 54), *Oryzopsis kingii,* and *Calamagrostis breweri* (map 41).

Alpine Grasses (maps 55 to 60).—There are only six truly alpine grasses in California, namely *Alopecurus aequalis* (map 60), *A. geniculatus* (map 59), *Glyceria californica* (map 57), *Koeleria cristata* (map 58), *Poa rupicola* (map 56), and *Festuca ovina* (map 55). The small number of alpines is remarkable and attests the youthfulness of the present alpine peaks. The species are specialized representatives, closely related to other members of their genera found on the lower slopes. None is endemic to the Sierra Nevada. Although isolated in the Sierran part of their range, specific differences have not been found in the Sierran material of *Koeleria cristata, Alopecurus geniculatus,* and *A. aequalis.*

Russell (1926) in his discussion of California climates states that "a narrow strip in the High Sierra ... [is] mapped as Tundra Climate.... This treeless alpine area differs sufficiently from the Microthermal area to justify its being placed in another type and there is little doubt that it meets the Köppen requirement of having all monthly means below 50°F." The true alpine grasses of California are closely confined to this narrow strip, at least in their high Sierran phase.

Grasses of the Northern Coast Ranges and Low to Middle Elevations in the Sierra Nevada (maps 61 to 81).—Still following the pattern of the grasses of northern California—that is, in the Coast Ranges to Monterey Bay, in the Sierra Nevada to Tulare County, and reappearing on the peaks of some southern California ranges—are a group whose center of abundance is in the northern Coast Ranges but which occur also in the Sierra Nevada, usually at low elevations. These grasses are again of northern origin, and in most cases represent different species of the same genera already mentioned. They tend to have narrower distributions, mostly within the limits of British Columbia to Idaho and southward to California—for example, *Melica geyeri* (map 61), *M. harfordii* (map 62), *M. californica* (map 63), *Festuca elmeri* (map 64), *Poa rhizomata* (map 65), *Panicum occidentale* (map 69), *Panicum pacificum* (map 70), *Stipa lemmoni* (map 72), *Festuca californica* (map 74), *Poa howellii* (map 75), *Bromus orcuttianus* (map 79), *B. vulgaris* (map 81), and *B. laevipes* (map 80). Only two—*B. grandis* (map 73) and *Melica torreyana* (map 76)—are actually confined to California.

Grasses of the Northern Coast Ranges (maps 82 to 99).—This final group occupying northern California contains species mostly endemic to California and usually of very restricted range. They are, again, all found northward from Monterey Bay, and thus form a strong contrast to the Coast Ranges from Monterey Bay southward to San Diego County wherein there is not known a single endemic of narrow or restricted range.

Characteristic of the Outer Coast Range and extending into Oregon are *Deschampsia caespitosa* var. *holciformis* (map 90), *Hierochloe occidentalis* (map 91), *Phalaris californica* (map 92), and *Calamagrostis nutkaensis* (map 98). Near the coast are the very rare *Hystrix californica* and *Agrostis californica* (map 99). More obviously specialized in habitat are *Poa napensis* (map 87) from a hot spring and *Calamagrostis crassiglumis* (map 95) from sphagnum bogs. Of the three species of *Pleuropogon*—*P. refractus* (map 84), *P. davyi* (map 85), and *P. californicus* (map 83)—two are endemic to California but fit the general scheme of northward relationship, for the only remaining known species in the genus are the very restricted *P. oregonus* and the circumboreal *P. sabinii.*

GREAT BASIN AND DESERT FLORAL UNITS IN CALIFORNIA

Desert Grasses (maps 100 to 126).—Species on nonalkaline soils in the desert include:

- Native perennials
  - *Aristida adscensionis* (map 118)
  - *Aristida californica* (map 119)
  - *Aristida divaricata* (map 120)
  - *Aristida fendleriana* (map 121)
  - *Aristida glabrata* (map 122)
  - *Aristida glauca* (map 123)
  - *Aristida parishii* (map 124)
  - *Aristida purpurea* (map 125)
  - *Aristida wrightii* (map 126)
  - *Bouteloua aristidoides* (map 114)
  - *Bouteloua barbata* (map 115)
  - *Bouteloua curtipendula* (map 116)
  - *Bouteloua gracilis* (map 100)
  - *Bouteloua rothrockii* (map 117)
  - *Hilaria rigida* (map 104)
  - *Panicum urvilleanum* (map 103)
  - *Poa bigelovii* (map 110)
  - *Triodia pulchella* (map 113)
- Native annuals
  - *Cenchrus echinatus*
  - *Cenchrus pauciflorus*
- Introduced annual
  - *Schismus barbatus*
- Weeds of cultivated land
  - *Eriochloa gracilis*
  - *Eriochloa aristata*
  - *Hordeum jubatum*
  - *Leptochloa filiformis* (map 101)
  - *Leptochloa uninervia* (map 102)
- Introduced or subtropical economic grasses
  - *Oryzopsis miliacea*
  - *Panicum miliaceum*
  - *Chloris gayana*
  - *Chloris virgata*
  - *Cynodon dactylon*

In connection with the flora of the extreme southern desert part of California, it is of interest to review the influence of the tremendously rich Mexican flora (Conzatti, 1943; Johnston, 1943) on that of California. The Mexican flora has roughly twice the number of native species that occur in California. This would indicate that so far as the grasses are concerned the relatively more stable geologic conditions that have existed in Mexico have been more favorable for the development and persistence of species than have the fluctuations in sea level, the glaciation, and the mountain unrest that have characterized California's past. Moreover, the sea and desert barriers that have existed through much of the past have been so effective in preventing a free interchange of species that, except for desert species and a few other exceptions the two floras are entirely distinct.

Although perhaps the best indicator species are tall, stiff *Hilaria rigida* (map 104) on the flats and low, fluffy *Triodia pulchella* (map 113) on the rocky slopes, in numbers *Aristida* (nine species) (maps 118 to 126) and *Bouteloua* (five species) (maps 100, 114 to 117) dominate the desert grass flora. This flora, in contrast to all the others found in California, has its strongest ties eastward and southward. *Bouteloua, Aristida,* and *Muhlenbergia* are well represented in the Mexican flora, each having one or two species in common with South American floras and in South America a second center of diversification. Clements (1934) postulates the "transformation of southeastern California from bunch-grass prairie to desert as a consequence of climatic changes" and describes *Hilaria rigida* as "derived directly from the adaptation of *Hilaria jamesii* (map 140) to a drying climate."

The close ties between the temperate floras of North and South America, already shown in the Sierran and Great Valley floras, are further demonstrated by the presence of these desert elements common to both hemispheres:

Aristida: compare the distributions of *A. ternipes* Cav. and *A. capillacea* Lam.
Bouteloua curtipendula (Griffiths, 1912)
Cenchrus echinatus (Hitchcock and Chase, 1920)
Cenchrus pauciflorus (Hitchcock and Chase, 1920)
Distichlis spicata (Hitchcock, 1927; Beetle, 1943*a*)
Muhlenbergia asperifolia (Hitchcock, 1927; Parodi, 1928; Beetle, 1943*c*)
Panicum urvilleanum (Hitchcock, 1935)
Sporobolus argutus (Hitchcock, 1927)
Stipa speciosa (Hitchcock, 1927)

In direct contrast to this North and South American relationship between the species of *Bouteloua, Muhlenbergia, Aristida,* and other genera is the situation in *Hierochloe.* The seven South American species of this genus (Parodi, 1941) show no immediate relationship to those of North America. They are concentrated in Magellanic floras (where *Muhlenbergia, Bouteloua,* and *Aristida* are entirely absent) and are linked to New Zealand by *Hierochloe redolens* ("Habita en el Ecuador, en el sur de Chile, y Argentina y en Nueva Zelandia"). Here is the strongest possible evidence in support of panantarctic dispersals.

Species of desert alkaline flats include:

Distichlis spicata var. *divaricata* (map 111)
Puccinellia parishii (map 112)
Sporobolus airoides
Sporobolus contractus (map 106)
Sporobolus cryptandrus

Great Basin Grasses.—The Great Basin has contributed a large number of grasses to the borders of California—that is, to the areas that lie eastward or southward of the Sierran crest, which has been almost without exception an effective barrier to their further dispersal. These Great Basin grasses include a number of alkali-tolerant species, *Poa ampla* (map 131), *Poa nevadensis* (map 132), *Puccinellia nuttalliana* (map 134), *Distichlis spicata* var. *stricta* (map 133), *Spartina gracilis* (map 137), and *Muhlenbergia asperifolia* (map 139). The other, alkali-intolerant species include a small but diverse Great Basin (and Colorado Desert) element:

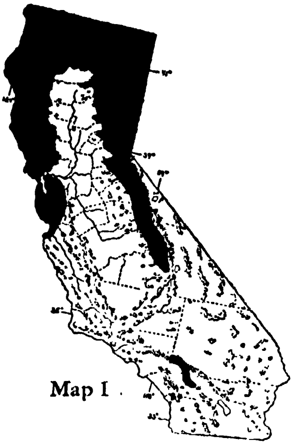
Agropyron smithii (map 143)
Agropyron spicatum (map 144)
Blepharidachne kingii (map 127)
Elymus cinereus (map 136)
Festuca kingii (map 128)
Hilaria jamesii (map 140)
Oryzopsis hymenoides (map 138)
Poa fendleriana (map 129)
Poa leptocoma (map 130)
Stipa comata
Stipa speciosa (map 142)
Stipa williamsii (map 141)
Triodia mutica (map 135)

These Great Basin species occupy the area of transition between the mountains and the desert. The group meets the desert grasses in central San Bernardino County but in general does not overlap their distribution.

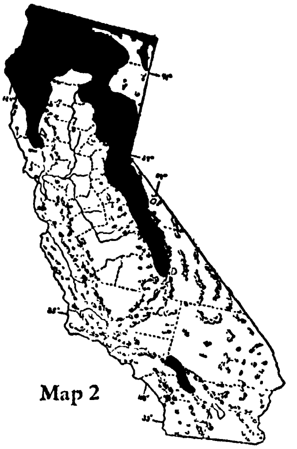
DISTRIBUTION MAPS FOR CALIFORNIA GRASSES

In the maps on the following pages, grasses with similar distributions are grouped together. Most of the grasses included are native species, but not all native species discussed in this paper are mapped. Those omitted would not affect the conclusions of the paper. The few introduced species mapped are ones that seem to have fully occupied the area to which they are adapted—that is, ones whose distribution has become relatively stable.

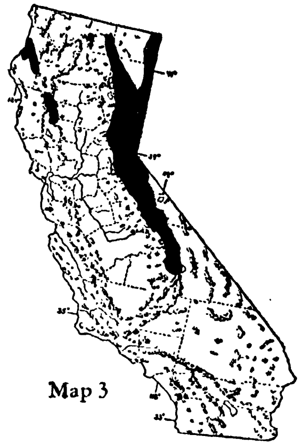
NORTHERN GRASSES THAT REACH THEIR SOUTHERN LIMIT IN CALIFORNIA


Map 1

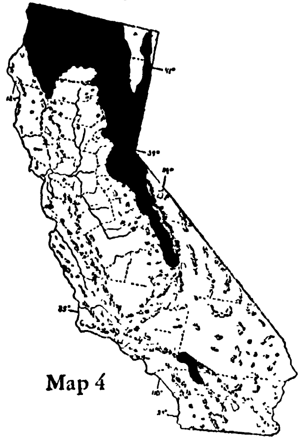
Poa pratensis


Map 2

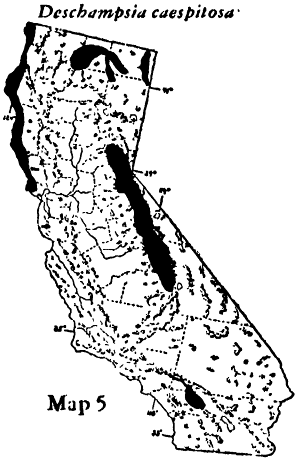
Deschampsia caespitosa


Map 3

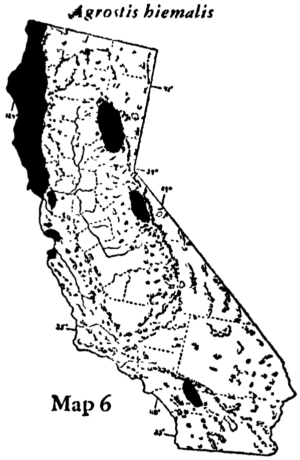
Agrostis hiemalis


Map 4

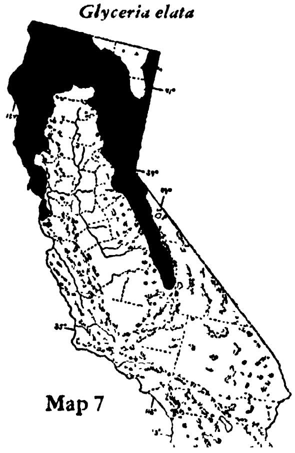
Glyceria elata


Map 5

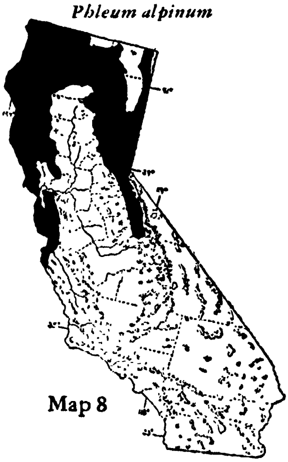
Phleum alpinum


Map 6

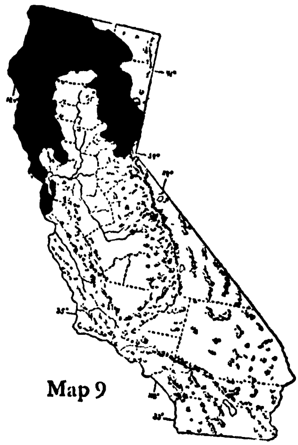
Trisetum cernuum var. *canescens*


Map 7

Glyceria pauciflora

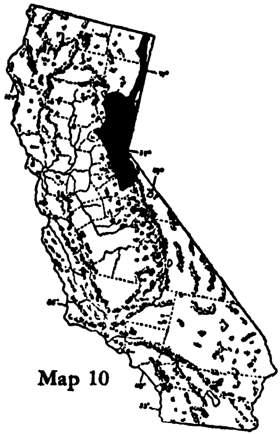

Map 8

Festuca rubra


Map 9

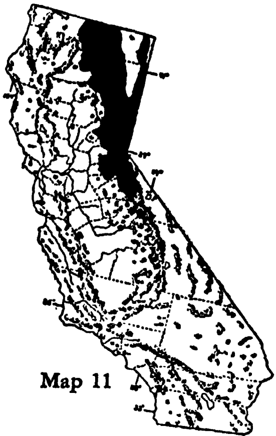
Melica subulata

NORTHERN GRASSES THAT REACH THEIR SOUTHERN LIMIT IN CALIFORNIA



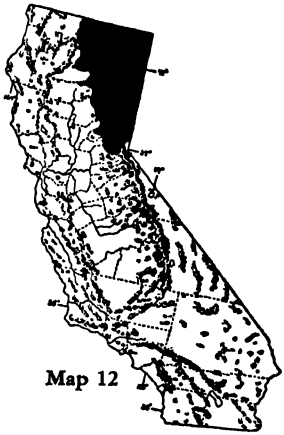
Map 10

Poa cusickii



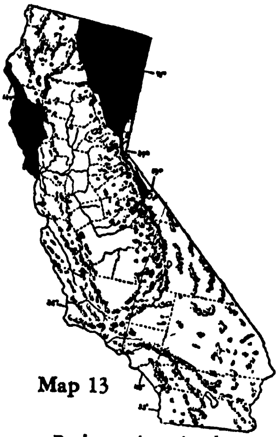
Map 11

Glyceria borealis



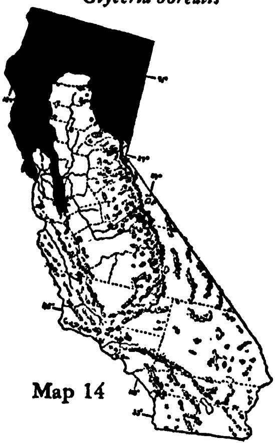
Map 12

Melica fugax



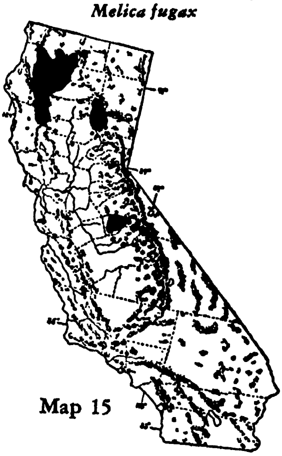
Map 13

Beckmannia syzigachne



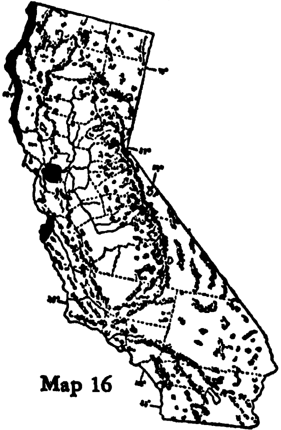
Map 14

Festuca idahoensis



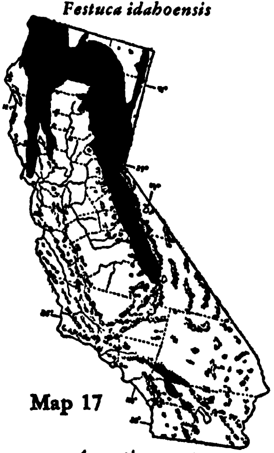
Map 15

Festuca subulata



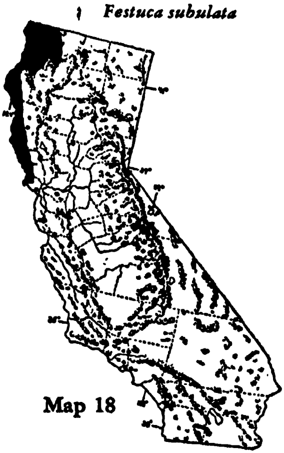
Map 16

Deschampsia caespitosa var. *arctica*



Map 17

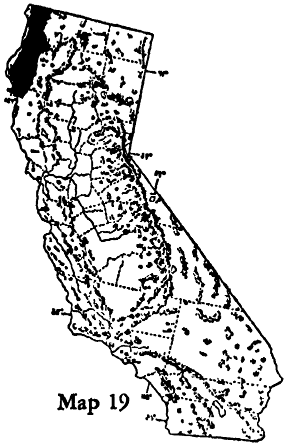
Agrostis exarata



Map 18

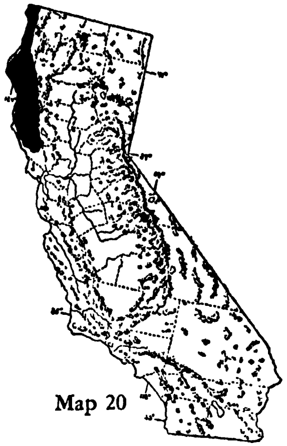
Trisetum cernuum

NORTHERN GRASSES THAT REACH THEIR SOUTHERN LIMIT IN CALIFORNIA



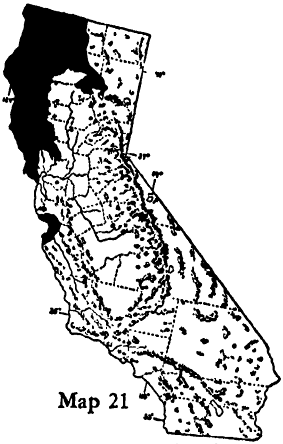
Map 19

Melica spectabilis



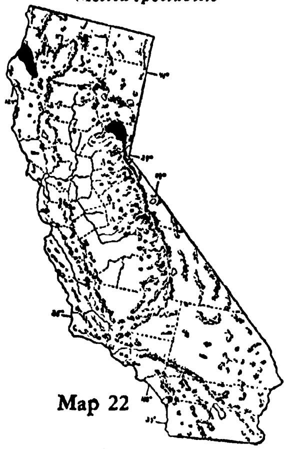
Map 20

Festuca subuliflora



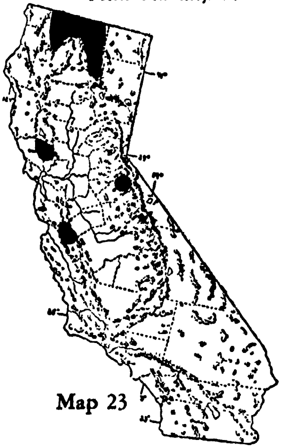
Map 21

Festuca occidentalis



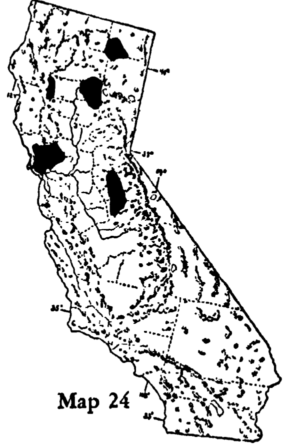
Map 22

Glyceria striata



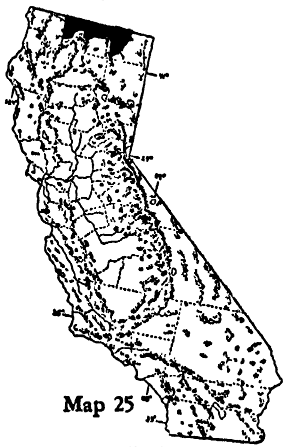
Map 23

Puccinellia distans



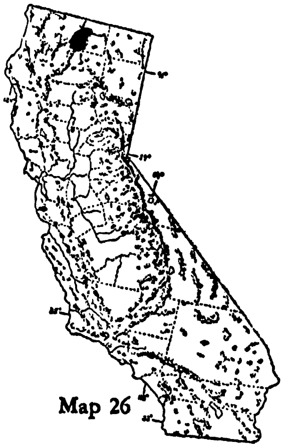
Map 24

Scribneria bolanderi



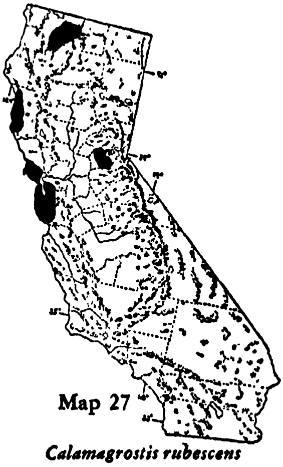
Map 25

Puccinellia lemmoni



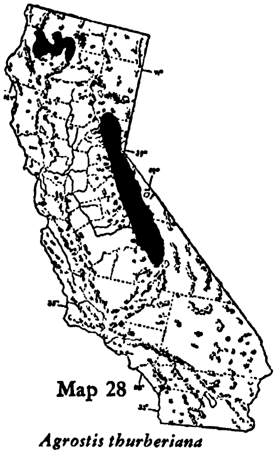
Map 26

Deschampsia atropurpurea



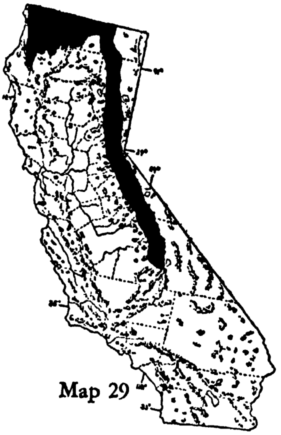
Map 27

Calamagrostis rubescens



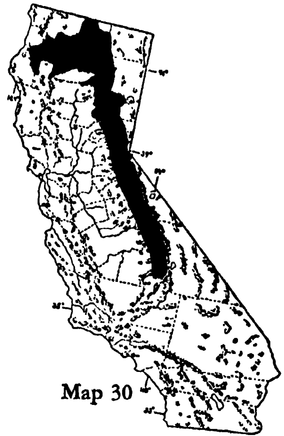
Map 28

Agrostis thurberiana



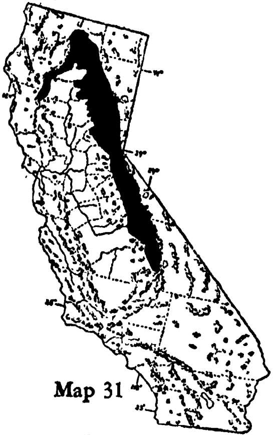
Map 29

Melica stricta



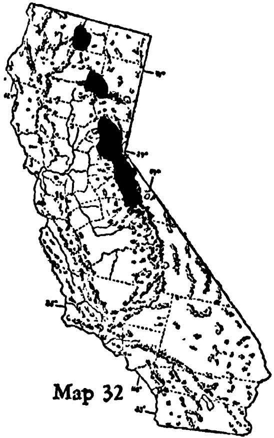
Map 30

Melica aristata



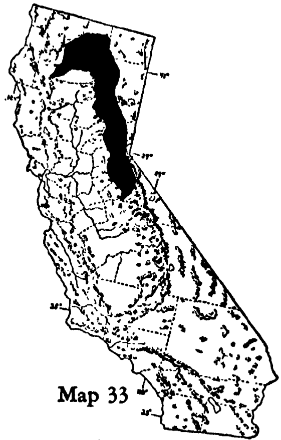
Map 31

Poa pringlei



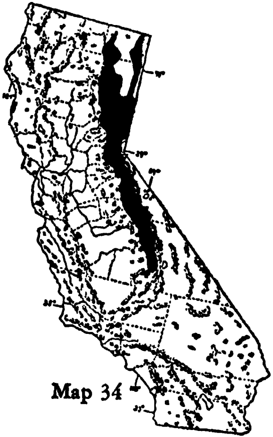
Map 32

Poa epilis



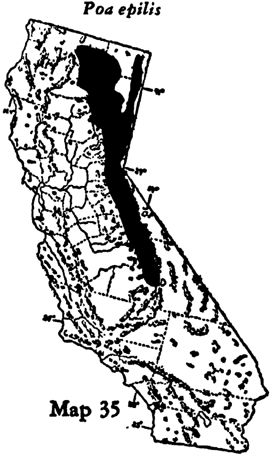
Map 33

Glyceria erecta



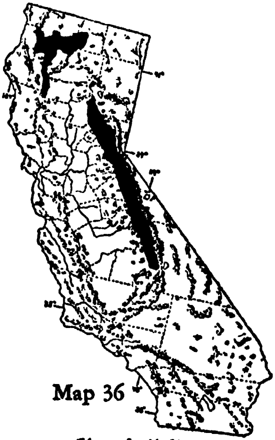
Map 34

Danthonia unispicata



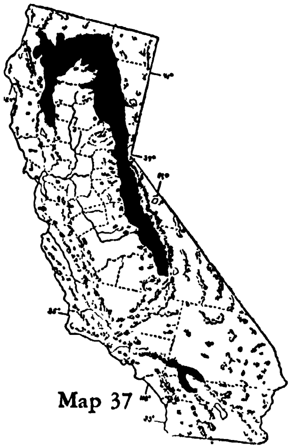
Map 35

Trisetum spicatum



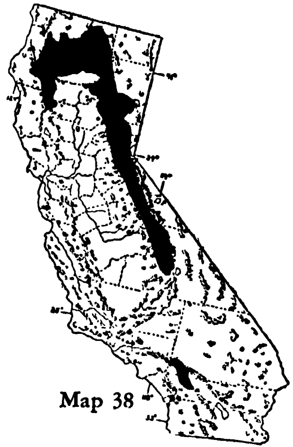
Map 36

Cinna latifolia



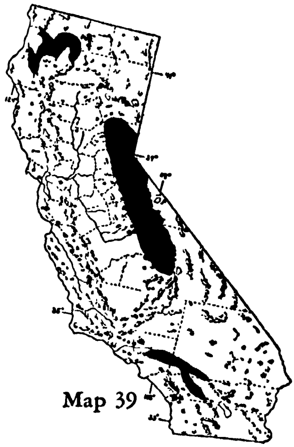
Map 37

Muhlenbergia filiformis



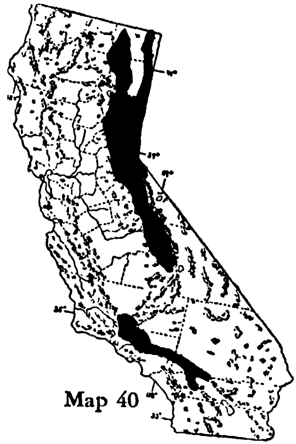
Map 38

Poa bolanderi



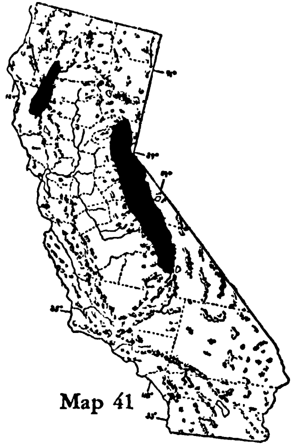
Map 39

Agrostis idahoensis



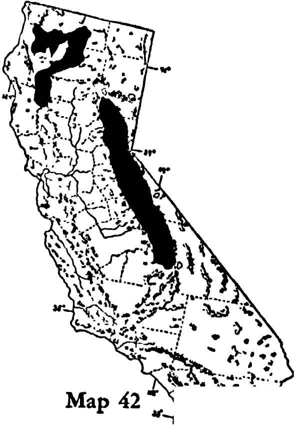
Map 40

Muhlenbergia richardsonis



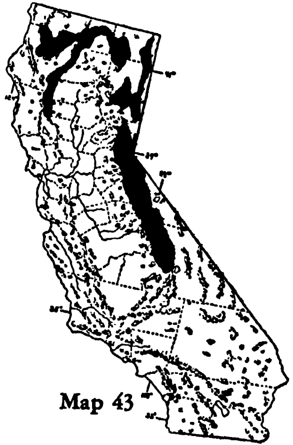
Map 41

Calamagrostis breweri



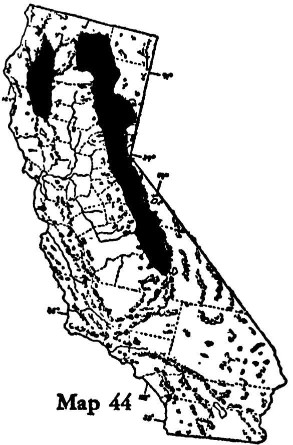
Map 42

Calamagrostis canadensis



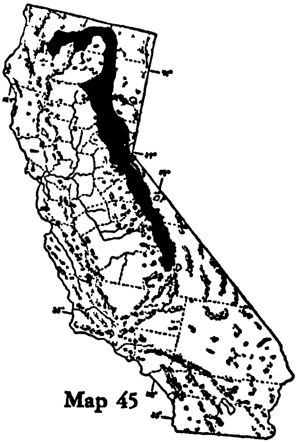
Map 43

Agrostis rossae



Map 44

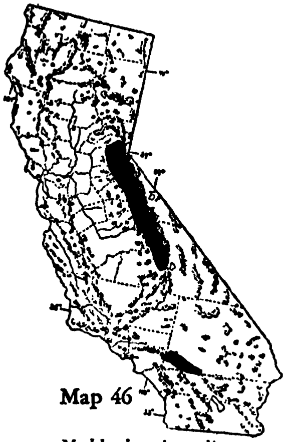
Melica bulbosa



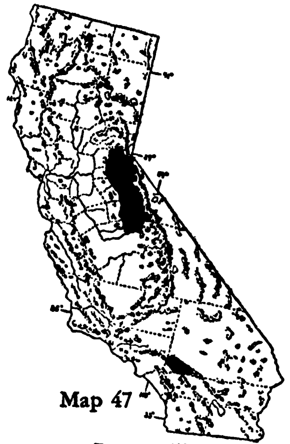
Map 45

Bromus suksdorfii

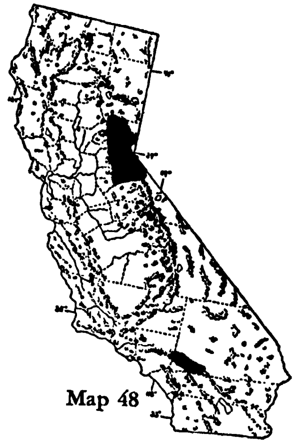
GRASSES OF HIGH ELEVATIONS IN THE SIERRA NEVADA


Map 46

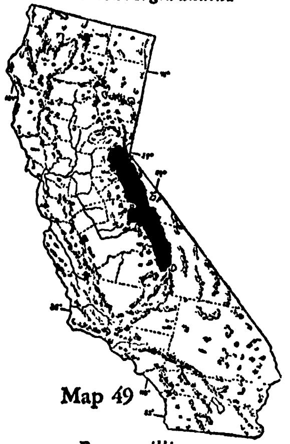
Muhlenbergia andina


Map 47

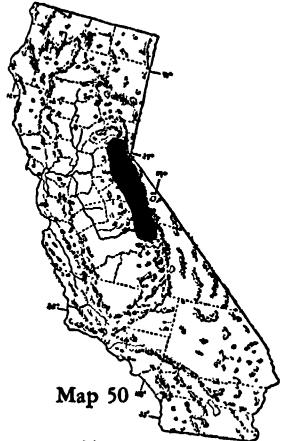
Bromus ciliatus


Map 48

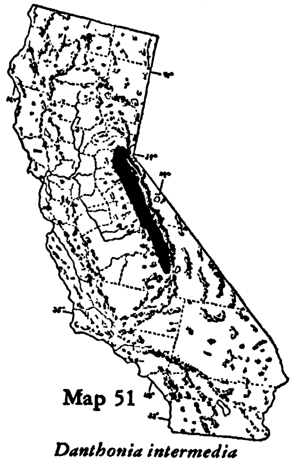
Festuca viridula


Map 49

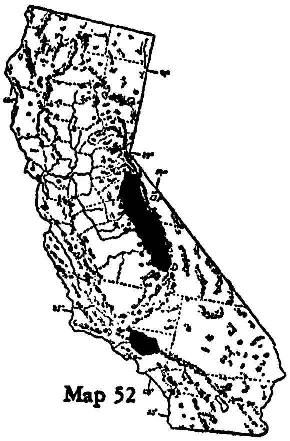
Poa gracillima


Map 50

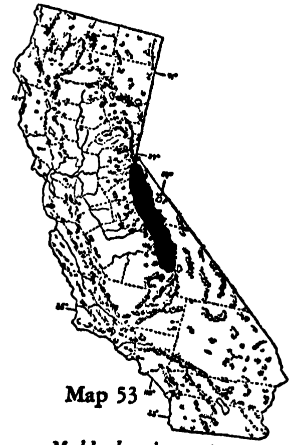
Trisetum wolfii


Map 51

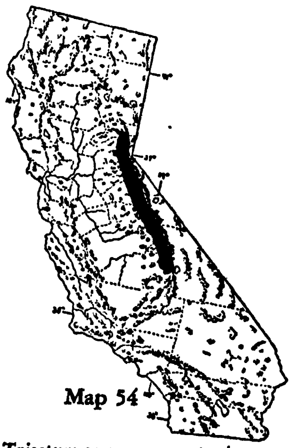
Danthonia intermedia


Map 52

Poa suksdorfii

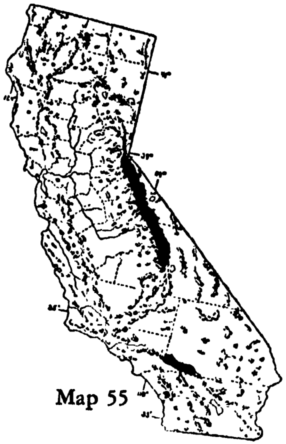

Map 53

Muhlenbergia montana

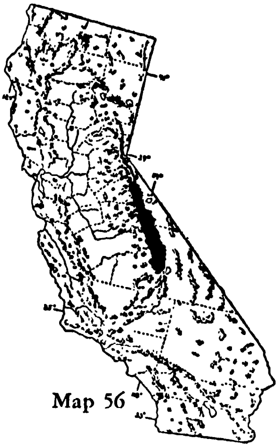

Map 54

Trisetum cernuum var. *projectum*

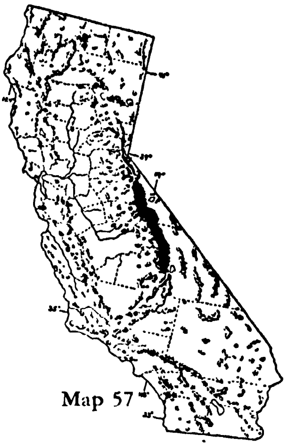
ALPINE GRASSES


Map 55

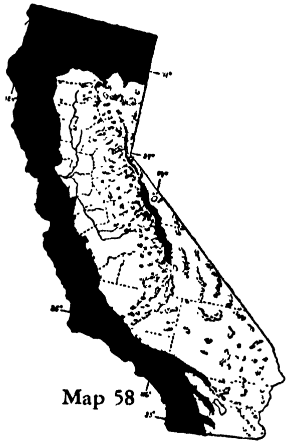
Festuca ovina


Map 56

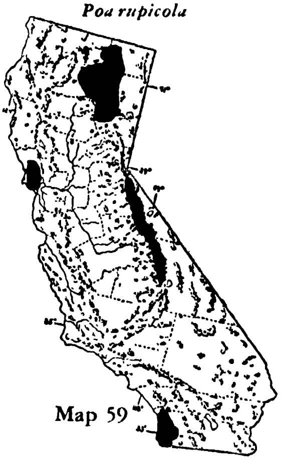
Poa rupicola


Map 57

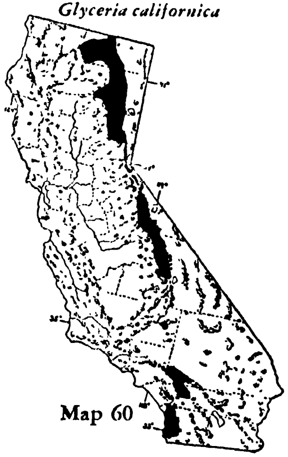
Glyceria californica


Map 58

Koeleria cristata

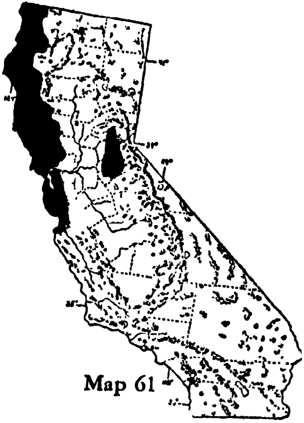

Map 59

Alopecurus geniculatus

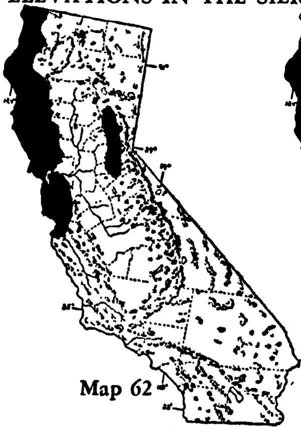

Map 60

Alopecurus aequalis

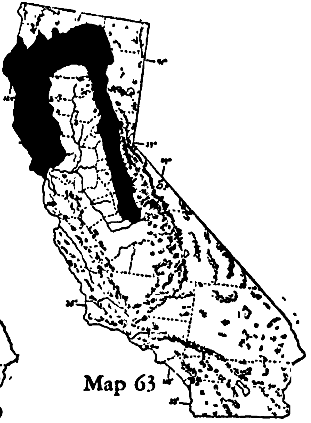
GRASSES OF THE NORTHERN COAST RANGES AND LOW TO MIDDLE ELEVATIONS IN THE SIERRA NEVADA


Map 61

Melica geyeri

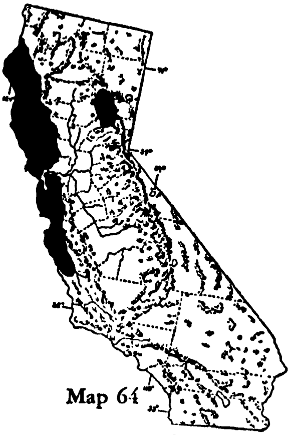

Map 62

Melica harfordii


Map 63

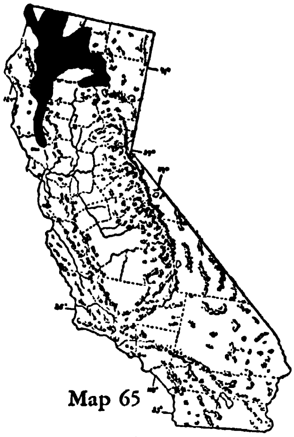
Melica californica

GRASSES OF THE NORTHERN COAST RANGES AND LOW TO MIDDLE ELEVATIONS IN THE SIERRA NEVADA



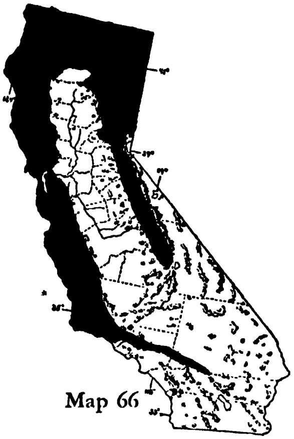
Map 64

Festuca elmeri



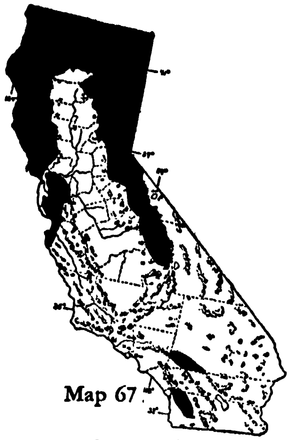
Map 65

Poa rhizomata



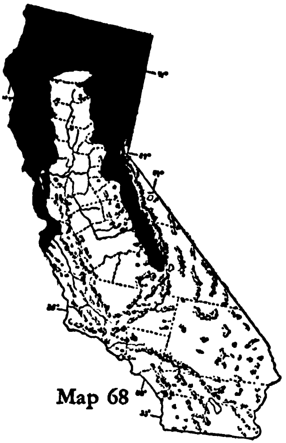
Map 66

Deschampsia danthonioides



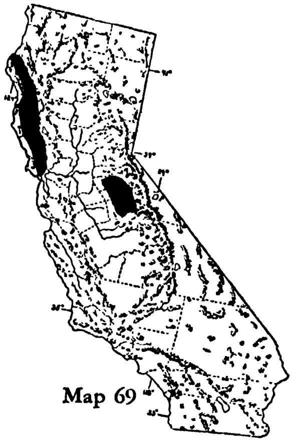
Map 67

Deschampsia elongata



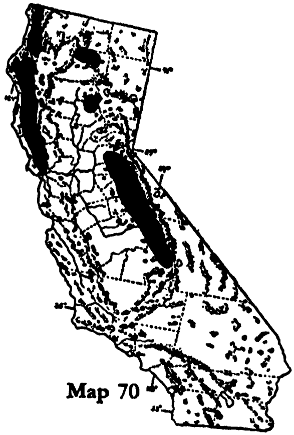
Map 68

Danthonia californica



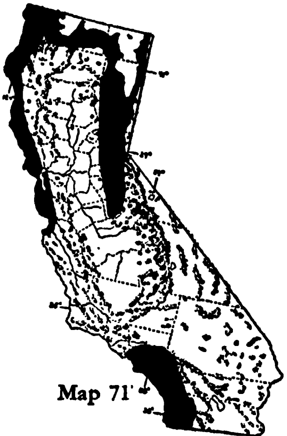
Map 69

Panicum occidentale



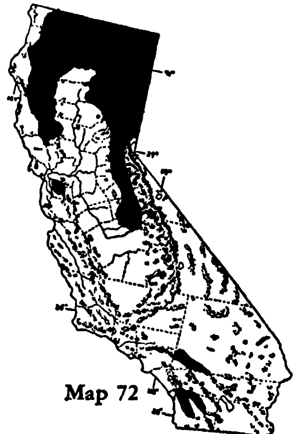
Map 70

Panicum pacificum



Map 71

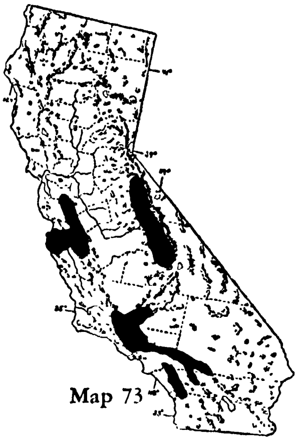
Agrostis diegoensis



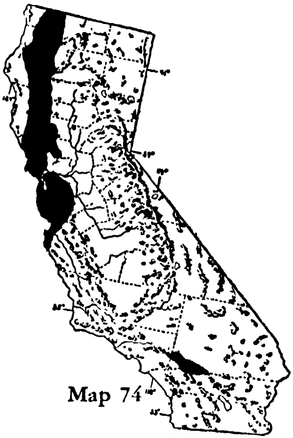
Map 72

Stipa lemmoni

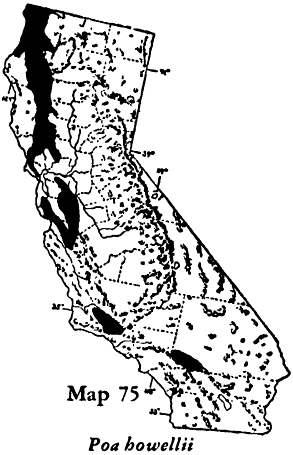
GRASSES OF THE NORTHERN COAST RANGES AND LOW TO MIDDLE ELEVATIONS IN THE SIERRA NEVADA


Map 73

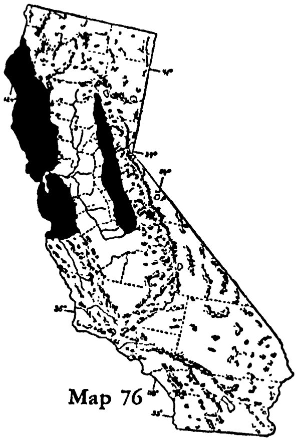
Bromus grandis


Map 74

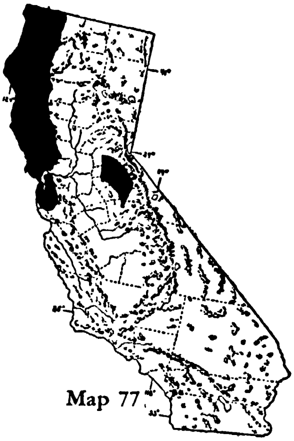
Festuca californica


Map 75

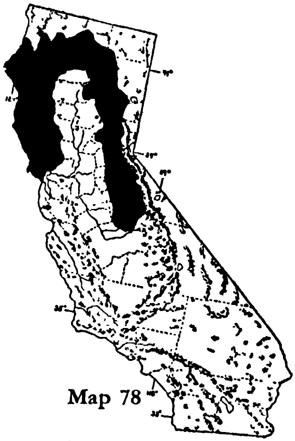
Poa howellii


Map 76

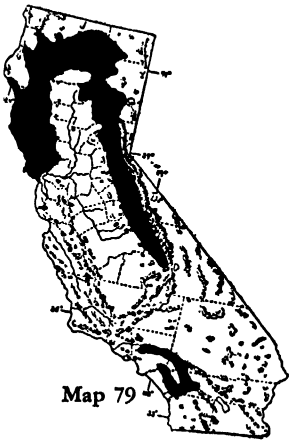
Melica torreyana


Map 77

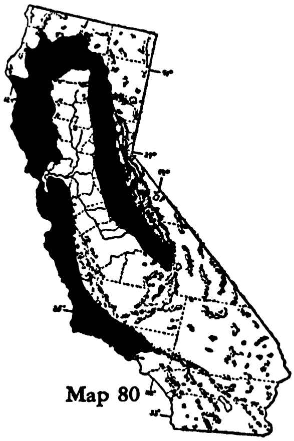
Bromus racemosus


Map 78

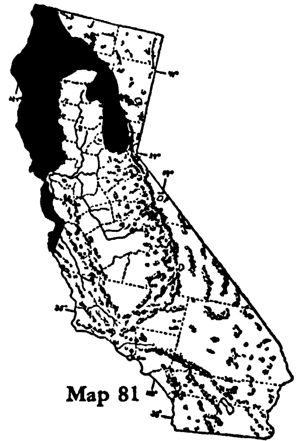
Bromus commutatus


Map 79

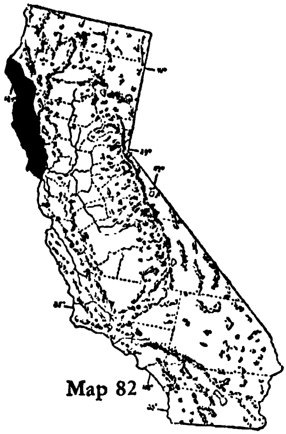
Bromus orcuttianus


Map 80

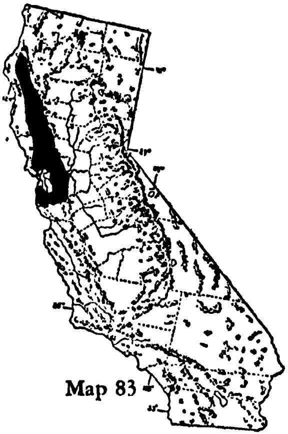
Bromus laevipes


Map 81

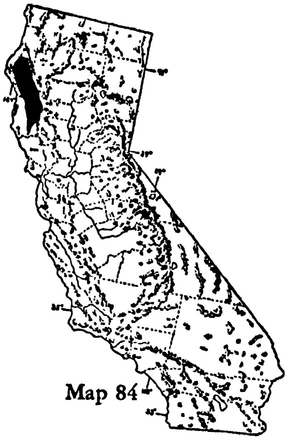
Bromus vulgaris


Map 82

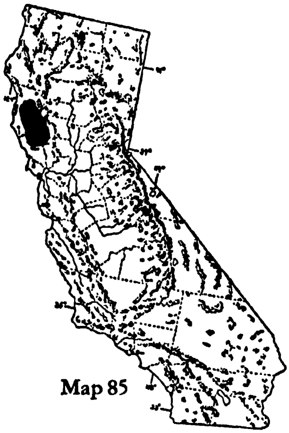
Poa trivialis


Map 83

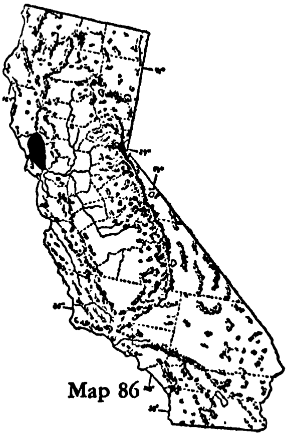
Pleuropogon californicus


Map 84

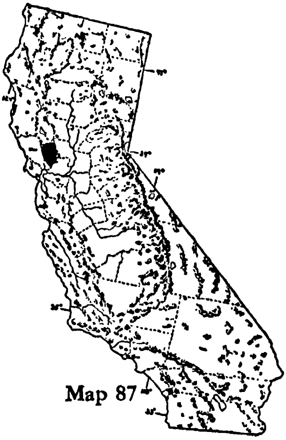
Pleuropogon refractus


Map 85

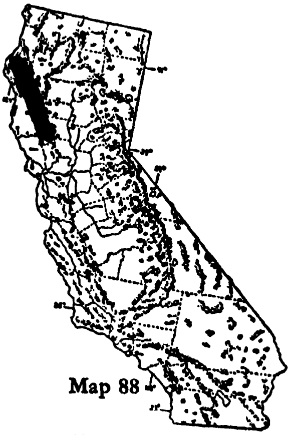
Pleuropogon davyi


Map 86

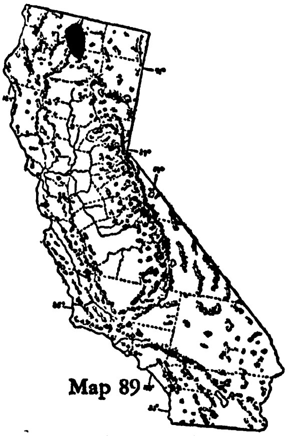
Glyceria leptostachya


Map 87

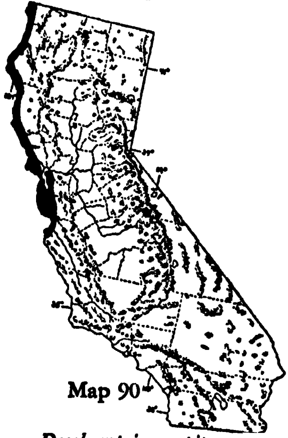
Poa napensis


Map 88

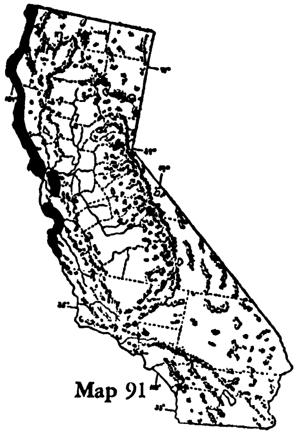
Glyceria occidentalis


Map 89

Glyceria cookei

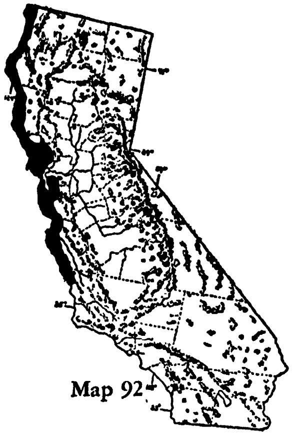

Map 90

Deschampsia caespitosa
var. holciformis



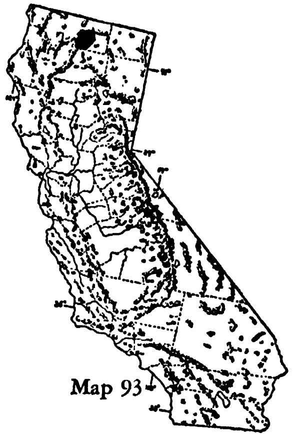
Map 91

Hierochloe occidentalis



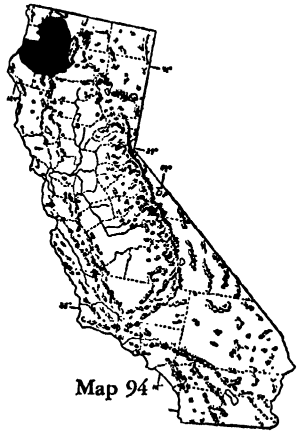
Map 92

Phalaris californica



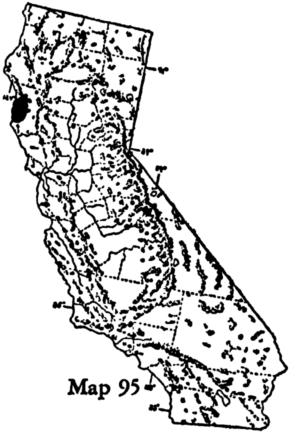
Map 93

Panicum shastense



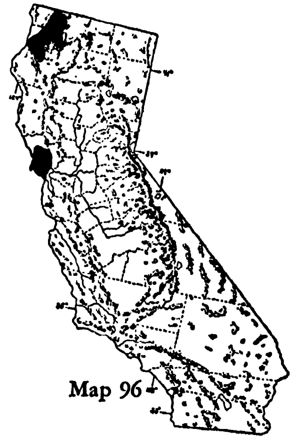
Map 94

Panicum scribnerianum



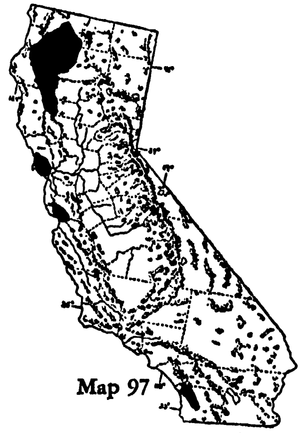
Map 95

Calamagrostis crassiglumis



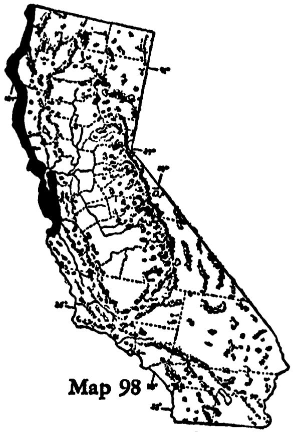
Map 96

Calamagrostis foliosa



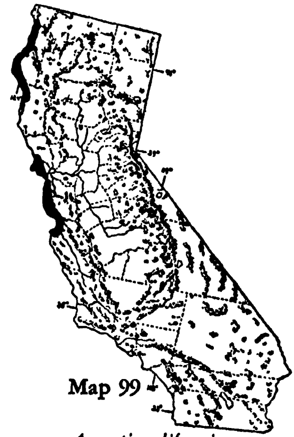
Map 97

Calamagrostis koelerioides



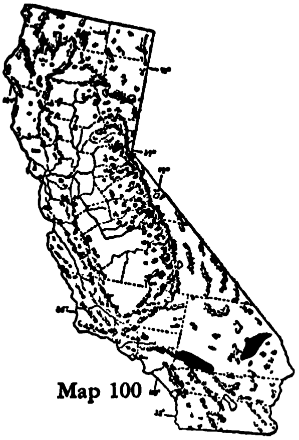
Map 98

Calamagrostis nutkaensis

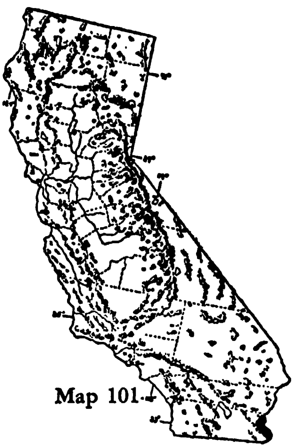


Map 99

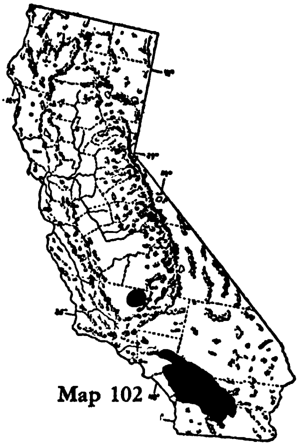
Agrostis californica


Map 100

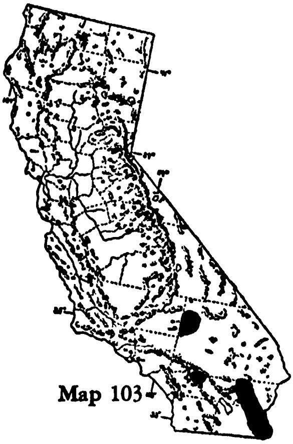
Bouteloua gracilis


Map 101

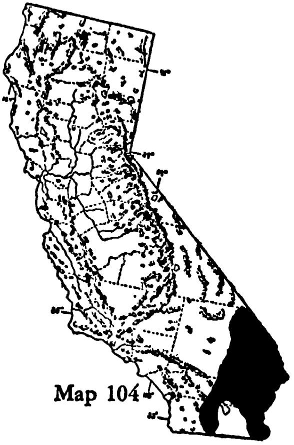
Leptochloa filiformis


Map 102

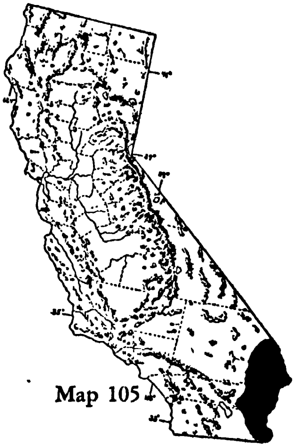
Leptochloa uninervia


Map 103

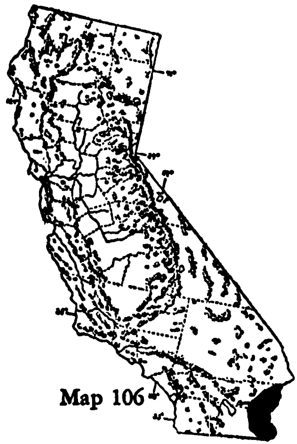
Panicum urvilleanum


Map 104

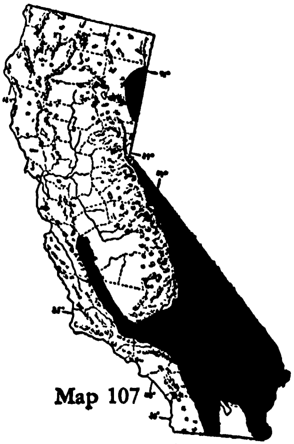
Hilaria rigida


Map 105

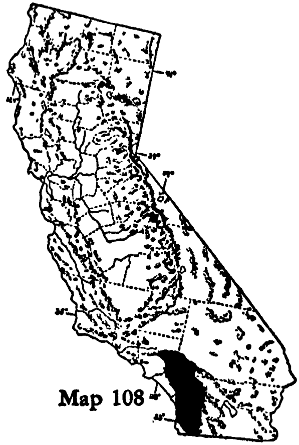
Muhlenbergia porteri


Map 106

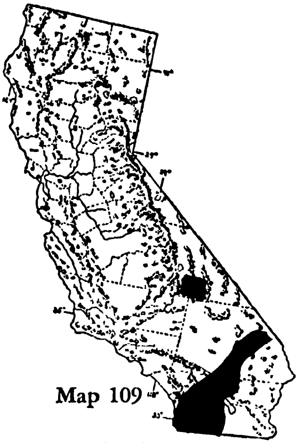
Sporobolus contractus


Map 107

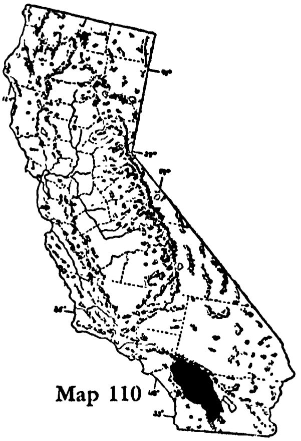
Stipa speciosa


Map 108

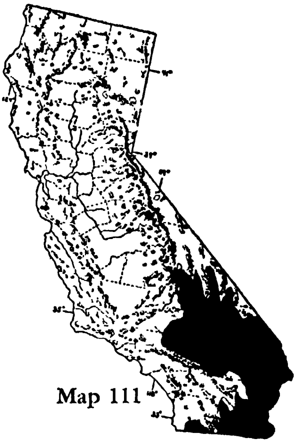
Muhlenbergia californica


Map 109

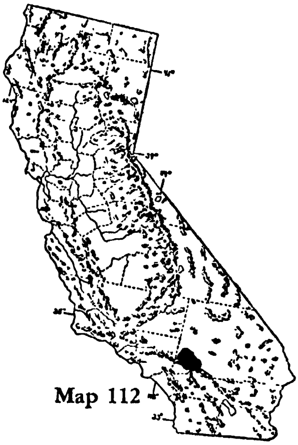
Melica frutescens


Map 110

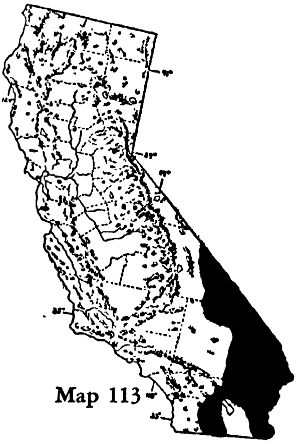
Poa bigelovii


Map 111

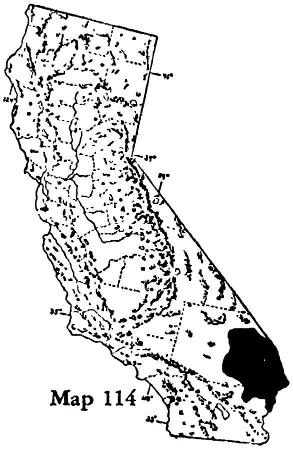
Distichlis spicata var. *divaricata*


Map 112

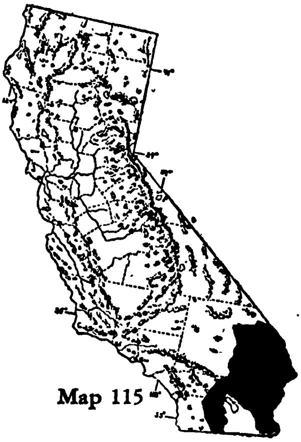
Puccinellia parishii


Map 113

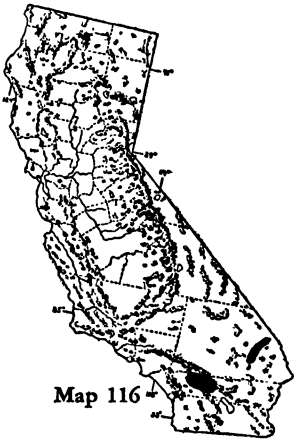
Triodia pulchella


Map 114

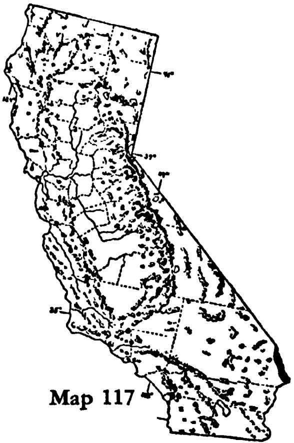
Bouteloua aristidoides


Map 115

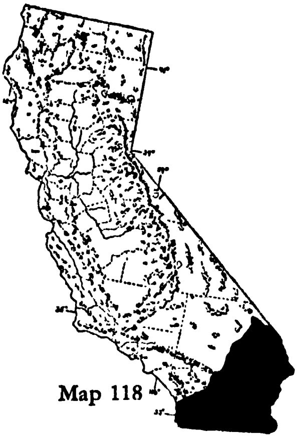
Bouteloua barbata


Map 116

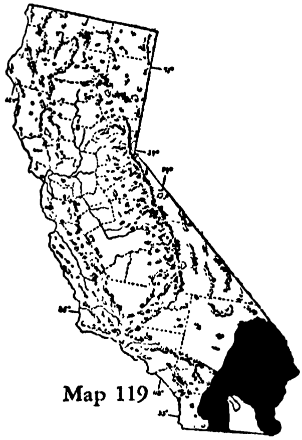
Bouteloua curtipendula


Map 117

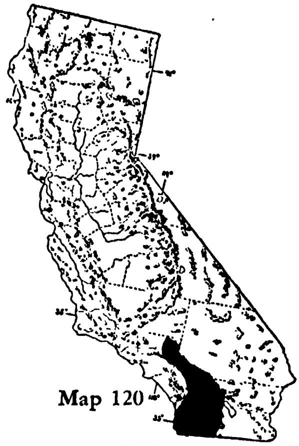
Bouteloua rothrockii


Map 118

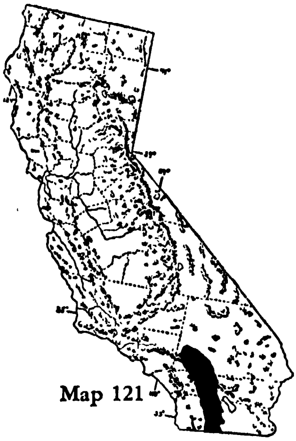
Aristida adscensionis


Map 119

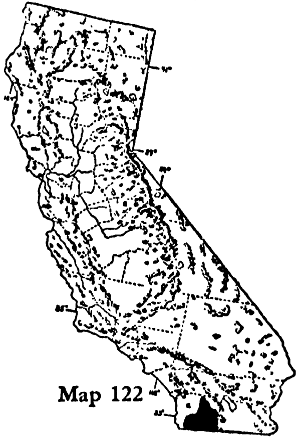
Aristida californica


Map 120

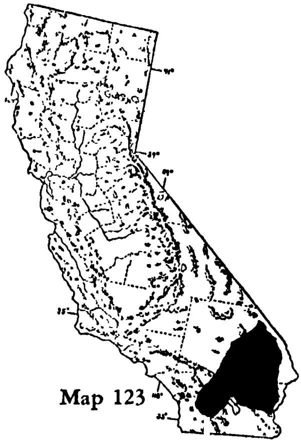
Aristida divaricata


Map 121

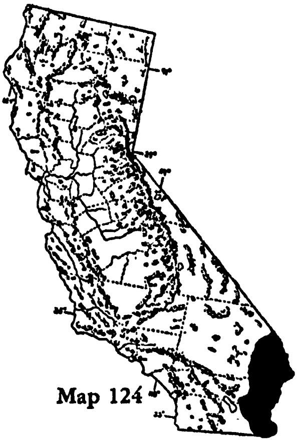
Aristida fendleriana


Map 122

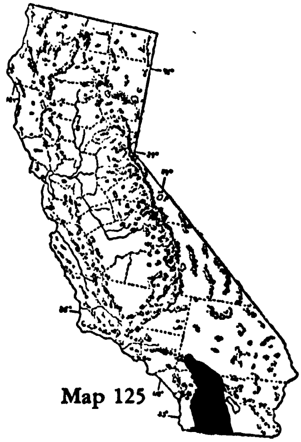
Aristida glabrata


Map 123

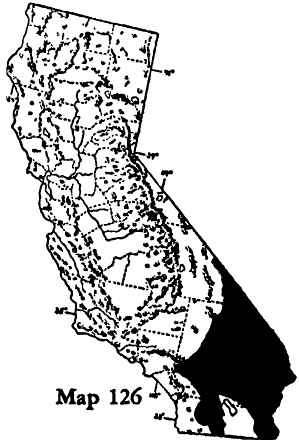
Aristida glauca


Map 124

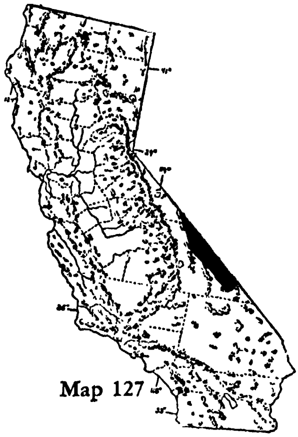
Aristida parishii


Map 125

Aristida purpurea

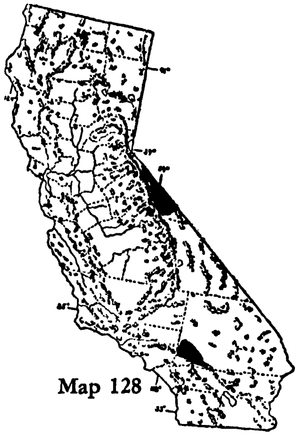

Map 126

Aristida wrightii



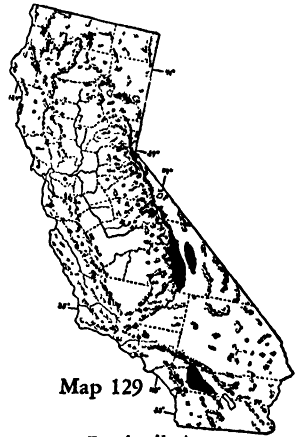
Map 127

Blepharidachne kingii



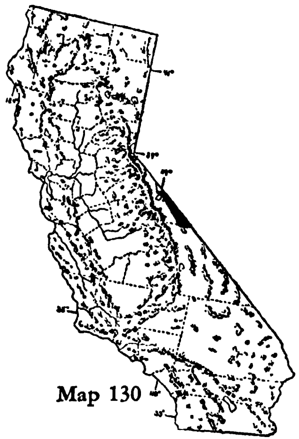
Map 128

Festuca kingii



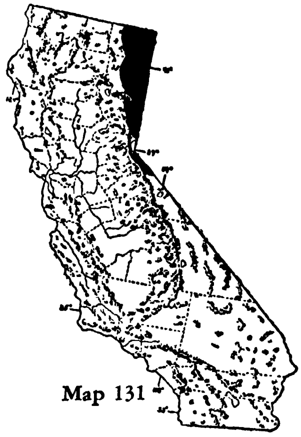
Map 129

Poa fendleriana



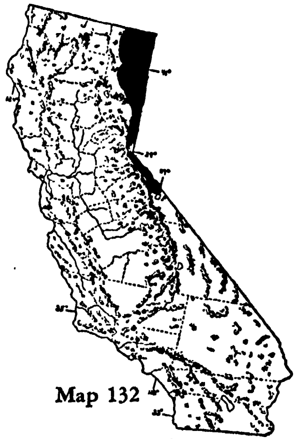
Map 130

Poa leptocoma



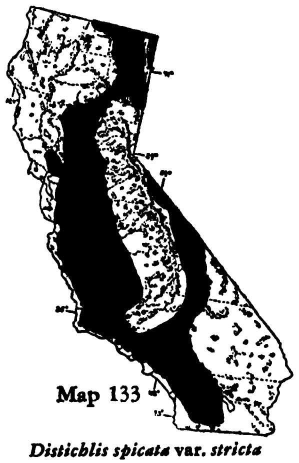
Map 131

Poa ampla



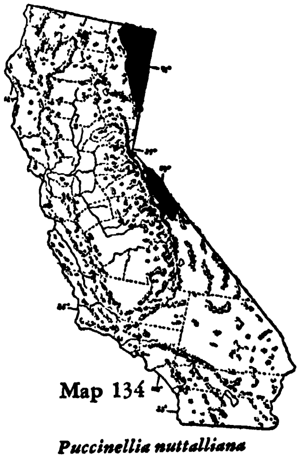
Map 132

Poa nevadensis



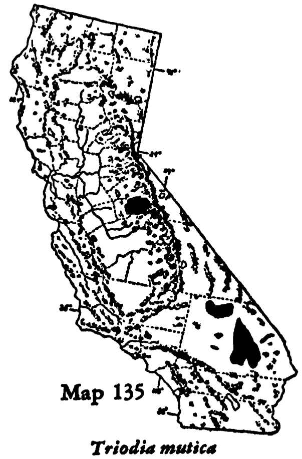
Map 133

Distichlis spicata var. *stricta*



Map 134

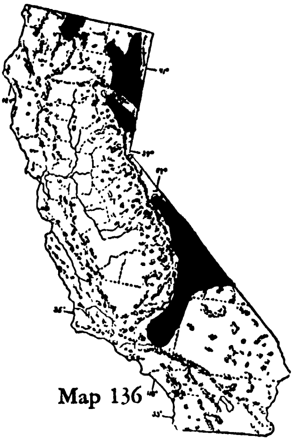
Puccinellia nuttalliana



Map 135

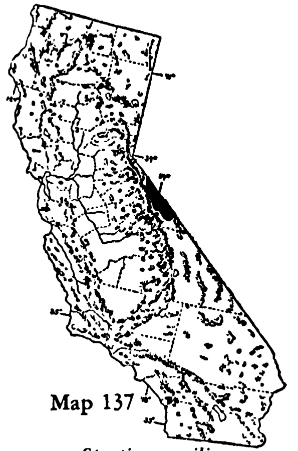
Triodia mutica

GREAT BASIN GRASSES



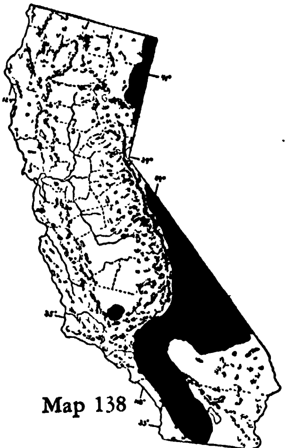
Map 136

Elymus cinereus



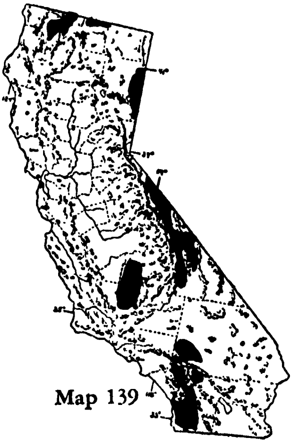
Map 137

Spartina gracilis



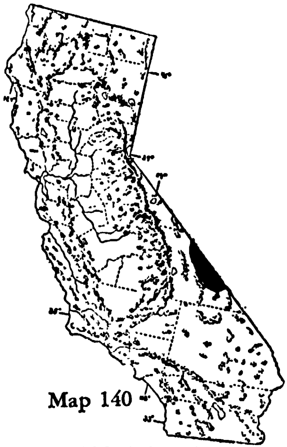
Map 138

Oryzopsis hymenoides



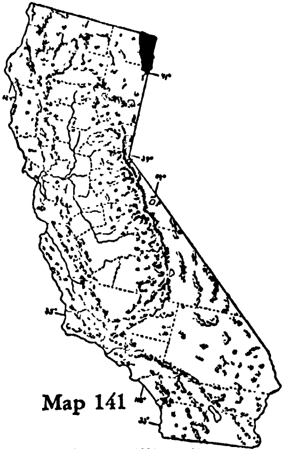
Map 139

Muhlenbergia asperifolia



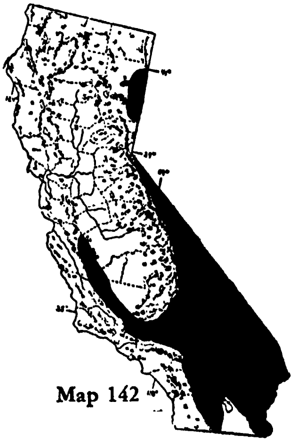
Map 140

Hilaria jamesii



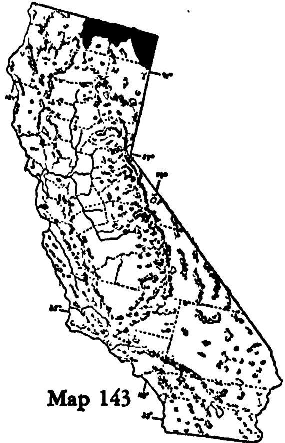
Map 141

Stipa williamsii



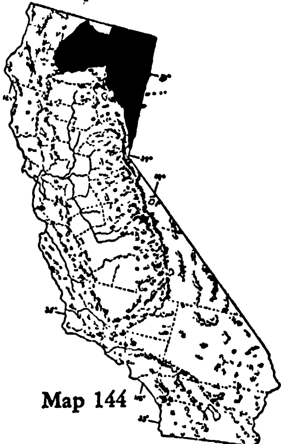
Map 142

Stipa speciosa



Map 143

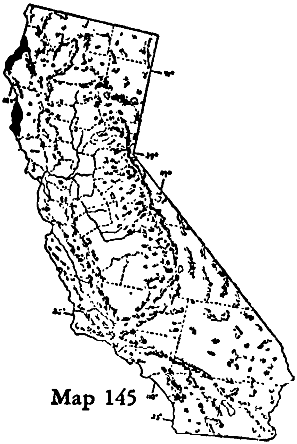
Agropyron smithii



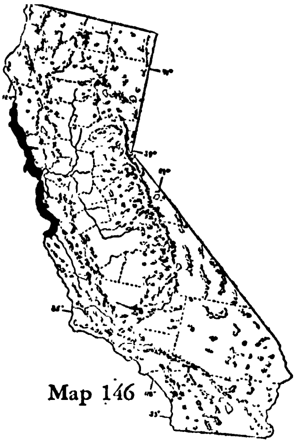
Map 144

Agropyron spicatum

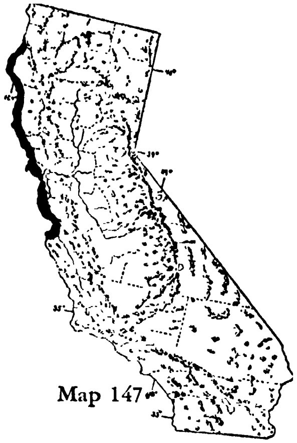
COASTAL GRASSES


Map 145

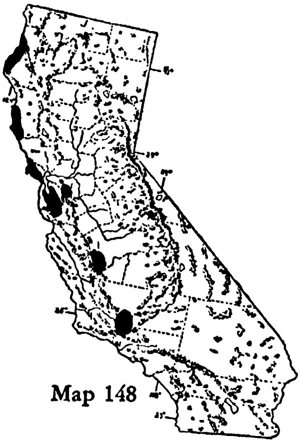
Poa macrantha


Map 146

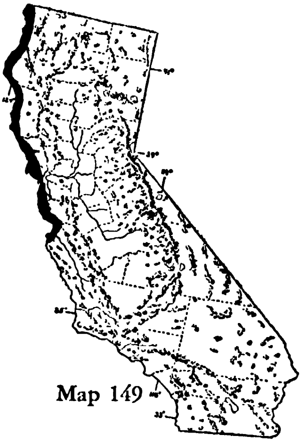
Poa douglasii


Map 147

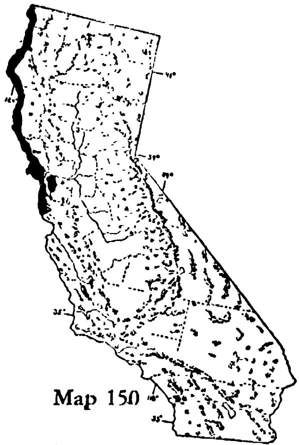
Poa unilateralis


Map 148

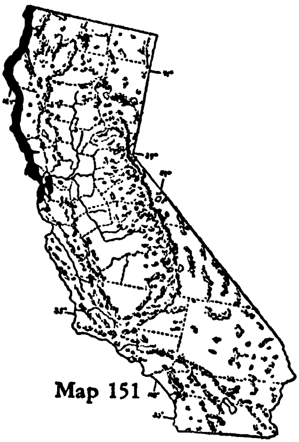
Puccinellia grandis


Map 149

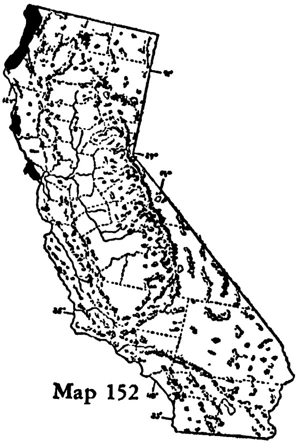
Elymus mollis


Map 150

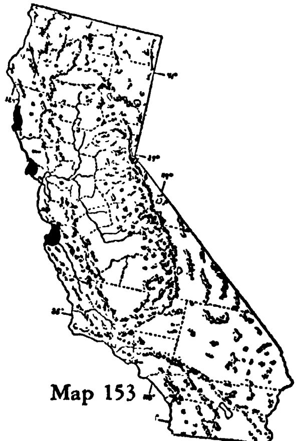
Elymus vancouverensis


Map 151

Elymus virescens

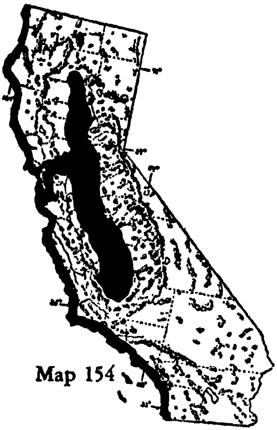

Map 152

Agrostis blasdalei

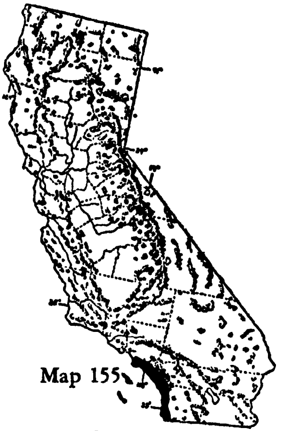

Map 153

Agropyron arenicola

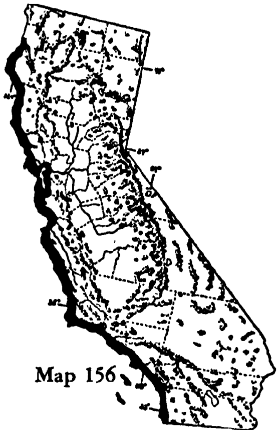
COASTAL GRASSES


Map 154

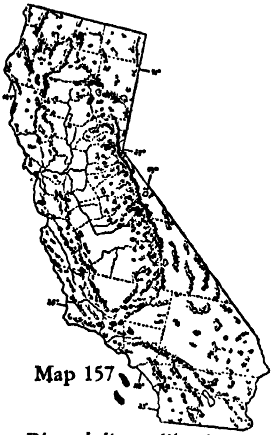
Phragmites communis


Map 155

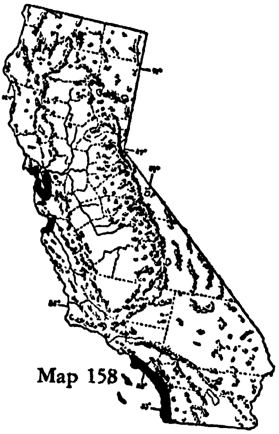
Monanthochloe littoralis


Map 156

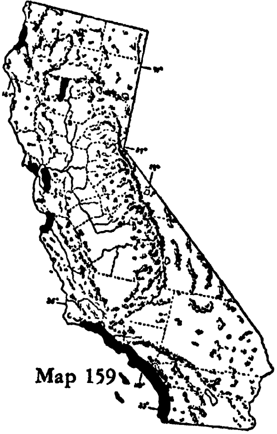
Distichlis spicata var. *stolonifera*


Map 157

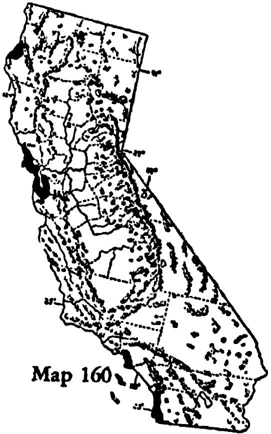
Dissanthelium californicum


Map 158

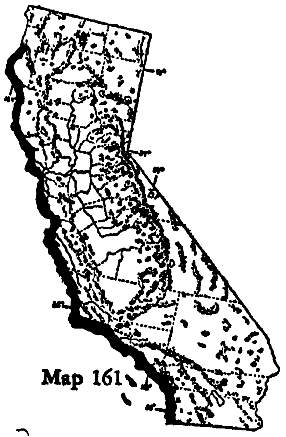
Lepturus cylindricus


Map 159

Pholiurus incurvus

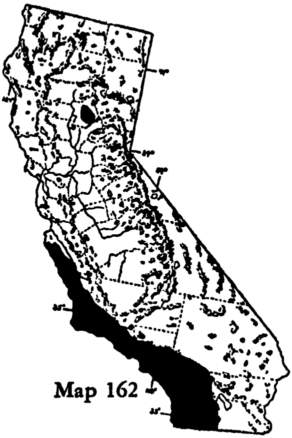

Map 160

Spartina foliosa

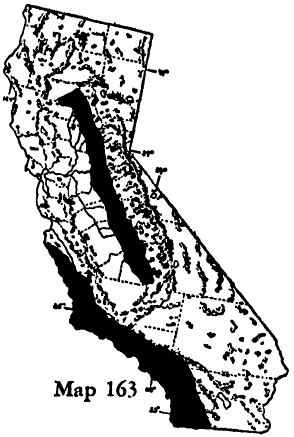

Map 161

Ammophila arenaria

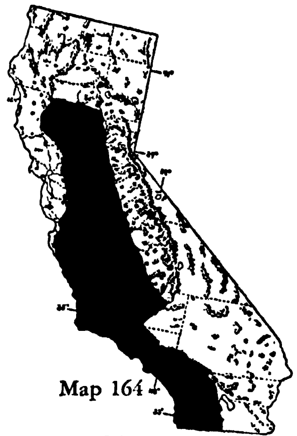
GRASSES OF THE SOUTHERN COAST RANGES AND GREAT VALLEY


Map 162

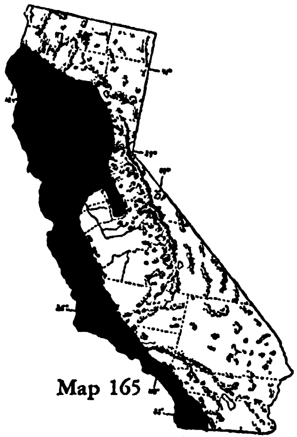
Muhlenbergia microsperma


Map 163

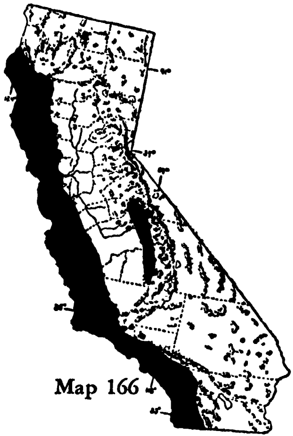
Muhlenbergia rigens


Map 164

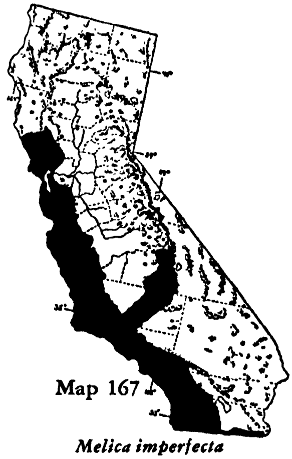
Stipa cernua


Map 165

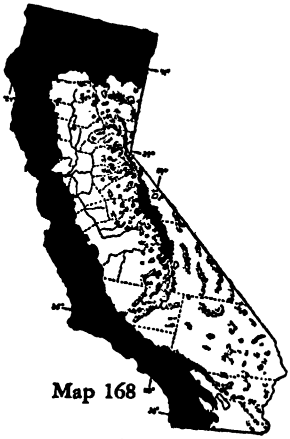
Stipa pulchra


Map 166

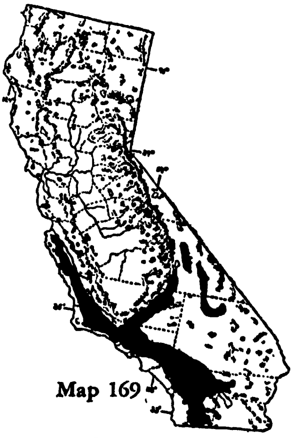
Stipa lepida


Map 167

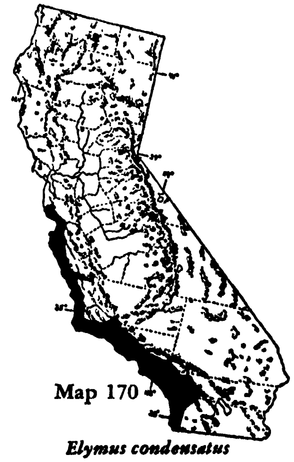
Melica imperfecta


Map 168

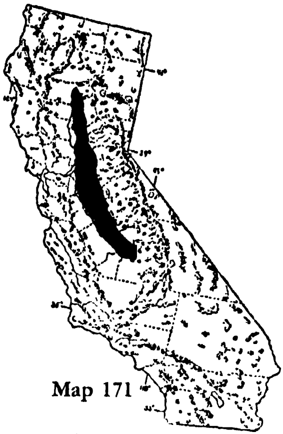
Koeleria cristata


Map 169

Stipa coronata

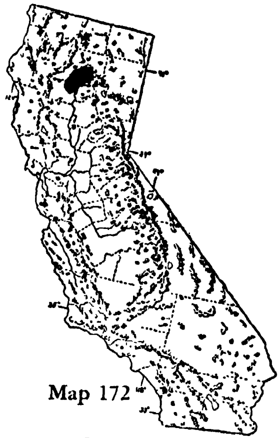

Map 170

Elymus condensatus



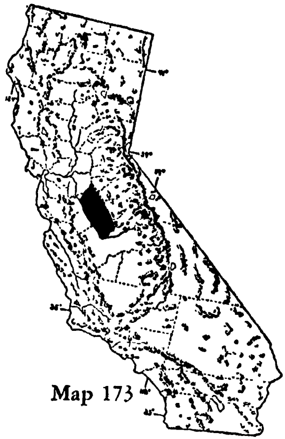
Map 171

Orcuttia greenei



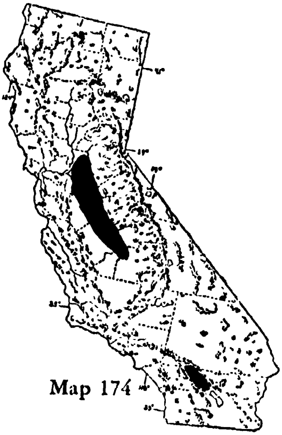
Map 172

Orcuttia tenuis



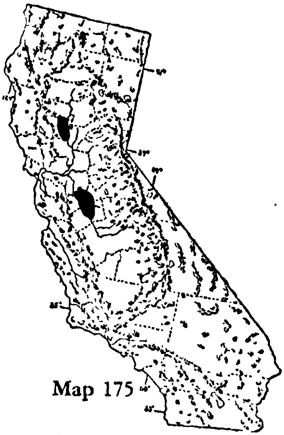
Map 173

Orcuttia pilosa



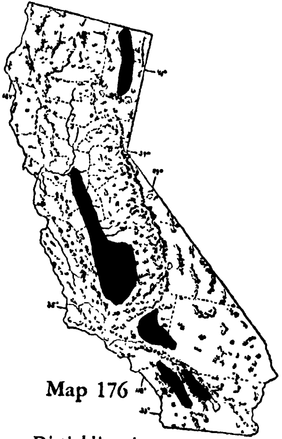
Map 174

Orcuttia californica



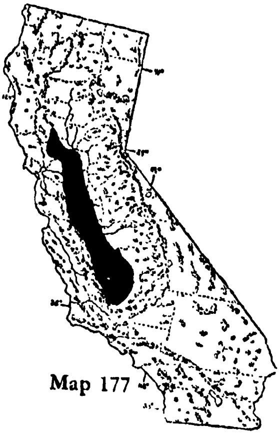
Map 175

Neostaphia colusana



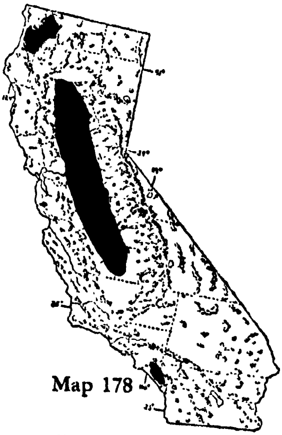
Map 176

Distichlis spicata var. *nana*



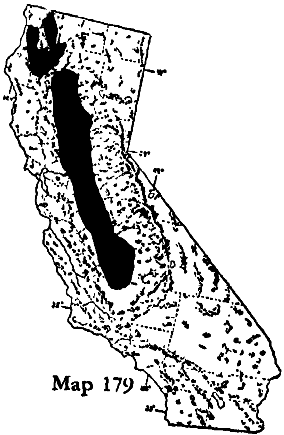
Map 177

Puccinellia simplex



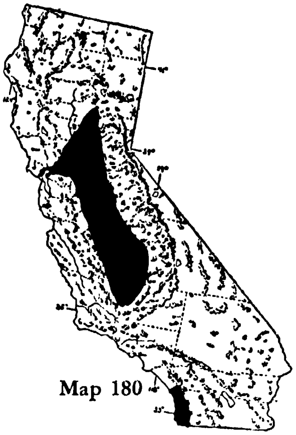
Map 178

Eragrostis hypnoides



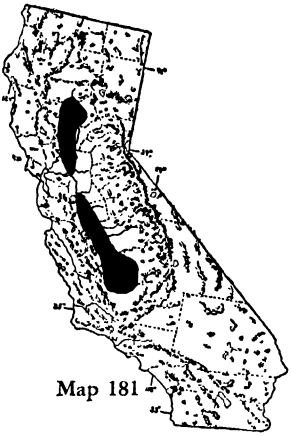
Map 179

Eragrostis orcuttiana



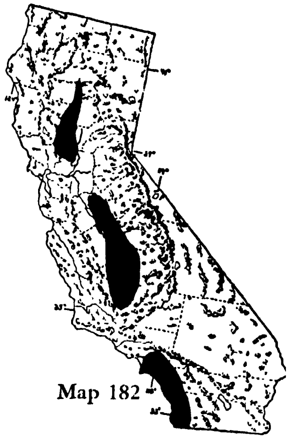
Map 180

Deschampsia danthonioides var. *gracilis*



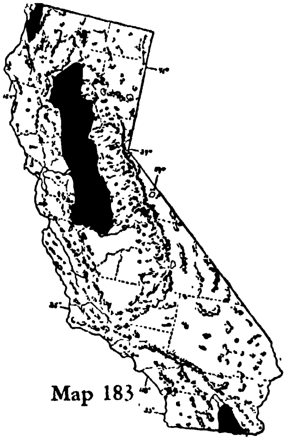
Map 181

Leptochloa fascicularis



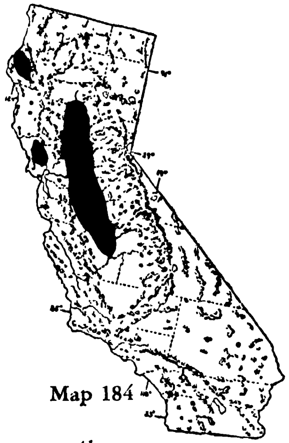
Map 182

Aristida hamulosa



Map 183

Aristida oligantha



Map 184

Alopecurus saccatus

THE CENTRAL VALLEY AND COASTAL FLORAL UNITS IN CALIFORNIA

ANALYSIS BY DISTRIBUTION

Coastal Grasses (maps 145 to 161).—Many of the coastal grasses are typical of the northern grasses discussed earlier—that is, they occur only north of Monterey Bay and belong to genera whose distributions are north temperate. They tend to be concentrated at such points along the coast as Humboldt Bay, Point Arena, Point Reyes, and the Monterey Peninsula. This is partly, but not wholly, due to restrictions of habitat. The disrupted ranges of the very rare *Agrostis blasdalei* (map 152) and *Agropyron arenicola* (map 153) illustrate this. Other representatives of northern genera that are restricted to coastal habitats include two closely related *Poa* species—*P. macrantha* (map 145) and *P. douglasii* (map 146)—the giant ryes, *Elymus mollis* (map 149) and *E. vancouverensis* (map 150), and the rare and little-known *Puccinellia grandis* (map 148).

Grasses which occur south of Monterey Bay (often as well as northward) are more typically of south-temperate genera and are often introduced; for example, *Lepturus cylindricus* (map 158), *Pholiurus incurvus* (map 159) and *Ammophila arenaria* (map 161).

Grasses of the Southern Coast Ranges and Great Valley (maps 162 to 170).—The native grass flora of the Coast Ranges from Monterey County south is very meager. The most typical are a trio of *Stipa* species, *S. cernua* (map 164), *S. pulchra* (map 165), *S. lepida* (map 166), and two *Muhlenbergia* species, *M. microsperma* (map 162), and *M. rigens* (map 163). These grasses may extend northward from Monterey Bay although, with the exception of *Koeleria cristata* (map 168), they do not reach the Oregon border. The abrupt termination in northward distribution at Monterey Bay of *Muhlenbergia microsperma, M. rigens, Stipa coronata* (map 169), and *Elymus condensatus* (map 170) emphasizes the importance of the area as the most critical in the Coast Ranges; see discussion of the southern termination of northern grasses.

Stipa cernua and *S. pulchra* occupy both the central valley and the southern Coast Ranges without a break in their distribution. This distribution, but with hiatus involving the western half of the valley and the Inner Coast Range, is found with *Andropogon virginicus* var. *hirsutior,*[4] *Muhlenbergia microsperma, M. rigens, Aristida hamulosa* (map 182), *Deschampsia danthonioides* var. *gracilis* (map 180), and *Sporobolus microspermus.*

Great Valley Grasses (maps 171 to 184).—The four species of *Orcuttia, O. greenei* (map 171), *O. tenuis* (map 172), *O. pilosa* (map 173), and *O. californica* (map 174), as well as *Neostaphia colusana* (map 175) lend a strong degree of endemism to the grass flora of the Great Valley. These grasses are all rare and specialized in habitat (see discussion under "Vernal Pools," p. 347–8). The grasses of the Great Valley are not found in the surrounding hills although they may occupy various of the low valleys which are adjacent to the Great Valley—for example, *Alopecurus saccatus* (map 184), *Eragrostis hypnoides* (map 178), and *E. orcuttiana* (map 179). All the species are annual except *Aristida hamulosa* (map 182) and *Distichlis spicata* var. *nana* (map 176).

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[4] The California phase of *Andropogon virginicus* L. should be treated as *Andropogon virginicus* var. *hirsutior* (Hack.) Hitchc., f. *tenuispatheus* (Nash) comb. nov. (*A. glomeratus tenuispatheus* Nash, *in* Small, Fl. S.E. U.S. 61. 1903; *A. tenuispatheus* Nash, *in* Gramineae. N. Amer. Fl. 17: 113. 1912.)

ANALYSIS BY HABITAT

Central California, as defined here, includes the Great Valley (San Joaquin and Sacramento valleys) and the surrounding upper Sonoran zone (to an elevation of about 3,000 to 4,000 feet) which is characteristically either open grassland, parklike savanna (the scattered trees usually oaks or digger pine), or chaparral. These habitats tend to be zonal in distribution (Piemeisel and Lawson, 1937), the alkali flats of the poorly drained bottomlands being bordered by grassland, and then chaparral or savanna on the slopes.

Open grass slopes dominate this area, and their typical golden color during most of the year is produced by dry grass straw. The first fall rains germinate the seeds of the annuals and renew the vegetative growth of the perennials. Duration of the growth depends upon the season; the hills stay green for the colder, wetter months, and in early spring begin to turn as the annuals rapidly mature and dry. The last seeds of the annuals are usually shed by the middle of April. Most of the perennials mature nearly as rapidly as the annuals, with the last of them maturing by June. Prolongation of the period of grazing on green feed at this period is one of the important range problems.

The only major break in the coastal hills occurs at San Francisco Bay where the great rivers, having converged, finally enter the ocean. This break considerably affects the climate of the region far into the valley but not sufficiently to form a barrier to plant dispersal. The annual rainfall in this central portion of the Great Valley reaches an average of 15 inches, but tapers off to the north and to the south. The Coast Ranges are lowest at San Francisco Bay (and Suisun Bay), and become higher and steeper northward and southward.

Coastal California is an area of highly specialized habitats. In some places chaparral, open grassland, or oak savanna approach the ocean, but more often there is an intervening strip, usually very narrow, of either coniferous woods (redwood, Monterey cypress, or Douglas-fir), or sand dunes or salt marsh. All owe their existence to, and are modified by, the proximity of the ocean, though in different ways—the salt in the marshes, the wind against the bluffs, the fog in the redwood belt.

All the habitats mentioned may occur also on the islands off the coast of California, to which the rare *Dissanthelium californicum* (map 158) is the only grass species known to be endemic. See Eastwood (1941) for a list of the species that have been collected on the islands.

Grasses of the Grassland and Savanna.—Parts of this portion of California appear to have been originally as nearly a climax type of grassland as the more extensive prairies of the interior of North America. The California grasslands were then dominated by bunch grasses liberally supplemented by erect perennials and a few stoloniferous perennials and interspersed with annuals. These areas differ in two respects from similar areas elsewhere in the world: (1) the identity of the perennial species; (2) the number and importance of the annuals. In character the California grassland is related to similar areas in eastern Oregon, eastern Washington and adjacent Idaho, and in western Montana. Together they comprise the Pacific bunch-grass area, as distinguished from the tall-grass and short-grass prairies of the Great Plains (McArdle, *et al.,* 1936). A far greater proportion of the species in the Pacific bunch-grass area, and even in the California area, trace their origins northward than is true in the tall-grass prairie (Weaver and Fitzpatrick, 1932) or the short-grass prairie, where a large southern element is usually dominant. Only *Koeleria cristata* occupies an important place in both the tall-grass and the Pacific bunch-grass floras.

The original appearance of the California grasslands is not a matter of historical record. Spanish settlers tilled the soil and grazed the hills for many years without caring to write about their surroundings in anything but the most general terms. As evidenced by adobe-brick studies (Hendry, 1931) such introductions as *Poa annua, Hordeum murinum,* and *Lolium multiflorum* made headway almost immediately. It is popularly supposed that the native bunch-grass perennials were far more abundant at the time and that they have since tended to disappear under the stress of cultivation, heavy grazing, and the competition of introductions. Since this area coincides with that principally occupied by California's enormous agricultural industry, such displacement is to be expected. Certainly in this portion of California no undisturbed remnants of the original flora remain (disturbance includes the effect of plant on plant as well as man on a plant community), and because of this its appearance is a matter for speculation only. Clements (1934) describes from observations made in 1918 the obliteration of "many hundred miles of a nearly continuous consociation of *Stipa pulchra,*" and the establishment of "the wild oats as the one great dominant throughout." There is no evidence, however, that any grass ever recorded for California has become extinct.

The number of strong contrasts between the California grassland and that of the Great Plains eliminates the possibility of any great similarity between the two. The California range is predominantly annual and therefore strongly fluctuating, whereas the Great Plains area is predominantly perennial and therefore potentially stable. The California range developed under a natural system of year-round grazing by deer, whereas the Great Plains area developed under a natural system of rotation grazing by buffalo. In California today introductions are predominant, and the state of the virgin range can no longer be considered a clue to its potential carrying capacity. In the Great Plains area natives are predominant today, and the state of the virgin range is an index to the carrying capacity. In California controlled grazing began around 1773, but on the Great Plains controlled grazing started about 1880. The two areas differ in the identity of both the native species and the introduced, often weedy species. They differ also in the degree of variability of the factors which influence the determination of grazing capacity. The difficulties in determining the carrying capacity of a given area are multiplied proportionately to: (1) the diversity of the vegetation; (2) the variability of the rainfall; (3) the variability of the climate; (4) the variability of slope; (5) the variability of the altitude; and (6) the diversity of soil types. Even within the counties of California all of the factors are present in a degree of variability not met in whole states in the Great Plains region.

The native bunch grasses (that is, nonstoloniferous types), whose conspicuous scattered clumps give the California part of the Pacific grassland its characteristic aspect, include:

Agrostis ampla
Andropogon virginicus var. *hirsutior*
Aristida hamulosa
Bromus carinatus
Bromus laevipes
Danthonia californica
Elymus condensatus
Elymus glaucus
Koeleria cristata
Melica californica
Melica imperfecta
Muhlenbergia rigens
Panicum pacificum and allies
Sitanion hystrix
Sitanion jubatum
Stipa cernua
Stipa lepida
Stipa pulchra

Successfully introduced stoloniferous perennials in this area are few because of moisture limitations (compare *Agrostis stolonifera, Phalaris arundinacea,* and *P. tuberosa* var. *stenoptera*). Of the grasses in this area, only *Elymus triticoides* is strongly rhizomatous.

Introduced perennials
Agrostis retrofracta
Agrostis tenuis
Danthonia pilosa
Lolium perenne

Native annuals
Agrostis exigua
Aristida oligantha
Bromus trinii
Eragrostis hypnoides
Eragrostis orcuttiana
Festuca megalura
Festuca microstachys
Festuca octoflora
Phalaris angusta
Scribneria bolanderi

Introduced annuals
Avena barbata
Avena fatua
Avena sativa
Briza maxima
Briza minor
Bromus mollis
Bromus commutatus
Bromus racemosus
Bromus rigidus
Bromus rubens
Elymus caput-medusae
Festuca dertonensis
Hordeum gussoneanum
Hordeum murinum
Lagurus ovatus
Lolium multiflorum
Lolium temulentum
Phalaris canariensis
Phalaris caroliniana
Phalaris minor
Phalaris paradoxa

The following introduced grasses are found in California only as weeds on disturbed or cultivated ground and usually at low elevations:

Aegilops triuncialis
Avena sterilis
Digitaria filiformis
Digitaria sanguinalis
Echinochloa colonum
Eleusine indica
Eragrostis cilianensis
Eragrostis poaeoides
Setaria lutescens
Setaria viridis

Southward from San Francisco Bay occur spontaneously a number of introductions of subtropical origin. Sometimes they occur on the open range, in rock crevices, in chaparral, or in oak woods, seemingly without regard to the habitats defined by the native vegetation. Unlike the introductions from the Mediterranean region, which are usually very abundant, make poor forage, and have harsh awns which cause mechanical injury to stock (for example, *Bromus* spp. and *Hordeum* spp.), these species are generally palatable and not mechanically injurious, but are much less aggressive.

Perennials	Annuals
Andropogon saccharoides	*Lamarckia aurea*
Imperata hookeri	*Schismus arabicus*
Pennisetum villosum	*Sorghum vulgare*
Sorghum halepense[5]	
Tricholaena rosea	

Phalaris platensis Henrard, an Argentine species, is reported to be very similar in characteristics to *P. lemmoni* (Henrard, 1938). The fact that *P. angusta* is considered native to both Chile and California, indicates that the genus is a real link between the floras of the two regions. Among the native California grasses of similar habitat additional links may be found in *Poa secunda* Presl, and *Bromus trinii* Desv. (Beetle, 1943*b*). This connection between a flora of California and one of temperate South America must be considered to have a history independent of the connections found in both the deserts and the mountains of the same regions (see p. 311 and 316). Campbell (1944) states that "species common to Chile and central southern California . . . may be explained as due to migration along the great mountain system of the Pacific Coast." It seems unlikely that the variously adapted species of grassland, desert, and mountain, all took the same route; thus desert species may have traveled a dry continental shelf similar to Lower California.

The genus *Panicum* (in number of described species one of the largest of all genera) as a whole and especially the subgenus Dichanthelium whose center of distribution is the coastal plain of the southeastern United States (Hitchcock and Chase, 1910), has found the mountains, deserts, and ocean surrounding California difficult barriers to traverse. Three perennial species (*P. scribnerianum, P. pacificum,* and *P. occidentale*), none of them common, are found at scattered localities, usually in at least semishade. A fourth, *P. thermale,* is known from the vicinity of the hot springs of Sonoma County. Thus the southeastern United States, recognized as a center of origin and dispersal for many nonboreal plants and animals of the greater part of the eastern United States (Adams, 1902), has made only a minor, occasional, accidental contribution to the flora of California; this holds true for the flora of California in general as well as for the grasses. It is apparent that the ocean to the west, the desert to the south, and the mountains to the east have long been barriers to the immigration of species to California from other floras. Only the north-to-south immigration route along the Coast Ranges and the Sierra Nevada into Oregon and Washington has been responsible for any appreciable contribution to the California grass flora from the Tehachapi Mountains northward.

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[5] At first glance, the California ranchers who have had trouble with Johnson grass may well be startled to find it listed as "less aggressive." Reference here is made, however, to the occurrence of these grasses on the dry, open range, a very different habitat from cultivated fields or irrigated pastures. In this connection it is interesting to note the apparently greater drought resistance of the bunch-grass type of perennial over stoloniferous or rhizomatous types. This accounts for the general rarity of introduced perennials on the range, as well as the general prevalence of introduced perennials in lawns and irrigated pastures.

Grasses of the Alkaline Flats.—Species of alkaline flats include:

Native perennials
- *Distichlis spicata* var. *nana*
- *Distichlis spicata* var. *stricta*
- *Puccinellia distans*
- *Puccinellia lemmoni*
- *Sporobolus airoides*

Native annuals
- *Hordeum pusillum*
- *Leptochloa fascicularis*
- *Puccinellia simplex*

The presence of any of the above is an indication of alkaline soil though *Distichlis spicata* var. *stricta* and *Puccinellia simplex* have the strongest tolerance. Alkaline soils are characterized by poor drainage, high evaporation, and an accumulation of soluble salts. These are not necessarily salts of alkaline reaction but include chlorides, sulfates, and carbonates and bicarbonates of sodium, potassium, calcium, and magnesium. Northward in the Great Valley the alkaline soils are all on the west side, but southward they are more common and occur on both sides of the valley.

With the exception of *Leptochloa fascicularis,* the species listed above represent typically north-temperate genera. Judging both from their centers of variation and the location of related species, the species of wide range listed above are probably immigrants to California. These include *Puccinellia distans, Hordeum pusillum,* and *Leptochloa fascicularis* (and probably also *Agropyron smithii,* which is of limited occurrence in northeastern California). This does not imply that they are not native in their occurrence, but simply that the impetus which resulted in their formation was elsewhere and that they subsequently found California a favorable environment. The remaining elements, on the other hand—varieties of *Distichlis spicata* and the species of *Puccinellia* which are narrow in range—may have been developed *in situ.* This appears to be particularly true of *Distichlis.* Here the origin of *Distichlis* from *Uniola,* or at least some ancestor with perfect florets, seems evident; dioeciousness is too rare in the Festuceae to be considered primitive where corroborative evidence is wholly lacking. Within *Distichlis* the species of larger plant body (for example, *D. texana*) are more characteristic of the Festuceae and are less specialized as to habitat; they might be regarded as precursors of the halophytic *D. spicata,* which has achieved a particularly wide range because of its adaptation to alkaline habitats.

Grasses of Aquatic Habitats.—The true aquatics include:

Native grasses
- *Alopecurus carolinianus*
- *Alopecurus howellii*
- *Alopecurus geniculatus*
- *Alopecurus saccatus*
- *Glyceria leptostachya*
- *Glyceria occidentalis*
- *Glyceria pauciflora*
- *Leersia oryzoides*
- *Paspalum distichum*
- *Phragmites communis*

Introduced perennials
- *Alopecurus pratensis*
- *Arundo donax*
- *Paspalum dilatatum*
- *Paspalum urvillei*
- *Setaria geniculata*

Introduced annuals
- *Agrostis semiverticillata*
- *Echinochloa crusgalli*
- *Polypogon interruptus*
- *Polypogon maritimus*
- *Polypogon monspeliensis*

All of the native perennial aquatics listed are of wide distribution, at least on the North American continent, except *Alopecurus howellii, A. saccatus,* and *Glyceria leptostachya,* which are confined to the northwestern corner of the United States from southern Washington to northern California. Both *Alopecurus* and *Glyceria* are north-temperate genera, and their California distributions fit the picture of predominantly northern origins proposed for species of other habitats.

Grasses of the Vernal Pools.—In vernal pools are found:

Introduced annuals
- *Crypsis aculeata*
- *Heleochloa schoenoides*

Native annuals
- *Agrostis microphylla* and its varieties
- *Deschampsia danthonioides* var. *gracilis*
- *Neostaphia colusana*
- *Orcuttia californica*
- *Orcuttia greenei*
- *Orcuttia pilosa*
- *Orcuttia tenuis*
- *Phalaris lemmoni*
- *Pleuropogon californicus*

The vernal pool represents an unusual habitat requiring considerable adaptation on the part of the plants inhabiting it. Water collects in pockets during the rainy season and, because of the high water table and poor drainage, remains as a small lake until late in the spring. When the water finally disappears as a result of evaporation, the annuals germinate (usually at a time when the surrounding vegetation has fully matured). Although their seeds have germinated in an aquatic habitat, these annuals must grow and mature in the hottest summer temperatures on dry, cracked soils. An odorous viscid pubescence reduces their transpiration rate. All are intolerant of salinity. None are perennial.

Both *Deschampsia danthonioides* var. *gracilis* and *Crypsis aculeata* are common; *Phalaris lemmoni* is frequent. The rest of the plants are rare, their habitat being limited and frequently disturbed by cultivation.

Orcuttia, Neostaphia, and *Pleuropogon,* all related according to Hoover (1941), are the rarest and most unusual genera of California grasses. Obscure as to relationship and highly specialized in habitat, the origin of the species of these three genera represents a difficult and as yet unsolved problem. The relationship between *Orcuttia* and *Neostaphia,* viscid annuals of unpleasant odor, is easier to see than any relationship between these two and *Pleuropogon. Pleuropogon,* a genus of five species and one variety (Benson, 1941), in its northern origin through *P. sabinii,* its generally perennial habit, and its aquatic habitat, not to mention the general morphological characters of inflorescence and spikelets, suggests a close relationship to *Glyceria.* The endemic species of *Pleuropogon, Orcuttia,* and *Neostaphia* agree with the Great Valley endemics of other families in belonging to distinctly California genera.[6] Hoover considers them to be endemics of recent (that is, derived) origin. They would then be called neoendemics, or initiates. This appears to be borne out by the fact that the valley floor was submerged in recent geological time. On the other hand, the isolated position of these genera (that is, the absence in the flora of close relatives, or links to other more common genera) suggests a long history. It may well be that these plants are restricted in range, not because they are very young nor because they are very old, but because in evolution they have become too selective as to habitat.

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[6] Hoover, R. F. Endemism in the flora of the Great Valley of California. A thesis submitted in partial fulfillment of requirements for the degree of Doctor of Philosophy, University of California. 175 p. 1937.

Chaparral Grasses.[7]—The most characteristic grass species of the chaparral belt include:

Southern
- *Melica frutescens*
- *Muhlenbergia microsperma*
- *Stipa parishii*
- *Stipa coronata*
- *Stipa lepida*

More northern
- *Calamagrostis densa*
- *Agrostis diegoensis*
- *Melica imperfecta*
- *Melica torreyana*

Only in the southern part of the state have grass species very successfully responded to the influence of the chaparral and become so restricted as seldom to be found outside the belt. *Stipa coronata* and *S. lepida* are common and conspicuous elements; the others are somewhat rare. Apparently these species originated in, or near, their present localities, for they have no near relatives in distant floras. According to Clements (1934) the grassland is accomplishing a "gradual encroachment" on the chaparral, but the two vegetational zones have existed "since the last great pulsation of the Pleistocene," as evidenced "by the universal absence of chaparral relicts."

Northward the pure chaparral has no characteristic grass species. Here are found *Calamagrostis densa* (obviously a narrower adaptation of *C. koelerioides*) and occasionally *Melica torreyana* and *Agrostis diegoensis.* Where there are especially dense stands of hard chaparral (for example, pure stands of *Adenostema* or *Arctostaphylos*) not only the grasses but nearly all other herbaceous species are generally forced out by the competition. Where the chaparral tends to be "soft," composed of mixtures of *Arctostaphylos, Quercus, Rhamnus, Ceanothus,* and similar plants, perennial species are frequent, but are species characteristic of oak woods or open ranges, such as *Stipa pulchra, S. californica, Melica californica, Sitanion hystrix, Poa secunda, Bromus carinatus, Elymus glaucus,* and *Koeleria cristata.* Introduced weedy annuals spring up whenever sufficient moisture is available (*Bromus rubens, B. mollis, Avena barbata, Gastridium ventricosum,* and *Aira caryophyllea*) but introduced perennials are absent. Not one of the twenty-one species listed by Sampson (1944) as "common to chaparral areas" is confined in its distribution to that area, but are the characteristic species of the oak woods and open grasslands.

Cooke (1940) in discussing *Stipa californica* in the Mount Shasta area, says:

> [It is] generally confined to the chaparral but also present throughout the transition zone and scattered through the Canadian zone. If the chaparral should be discussed as a grass association it could be called an *Elymus glaucus–Stipa californica–Bromus carinatus* association.

Grasses of Coastal Bluffs and Sand Dunes.—Along the strand the characteristic species include:

Native
- *Agropyron arenicola*
- *Agrostis blasdalei*
- *Agrostis californica*

Native (*continued*)
- *Agrostis pallens*
- *Calamagrostis foliosa*
- *Elymus mollis*

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[7] For a discussion of the ecology of and distribution of the chaparral belt, see Sampson (1944) and Jepson (1925).

Native (*continued*)
- *Elymus vancouverensis*
- *Elymus virescens*
- *Poa confinis*
- *Poa douglasii*
- *Poa macrantha*
- *Poa unilateralis*

Introduced annuals
- *Holcus lanatus*
- *Holcus mollis*

Introduced perennials
- *Agropyron junceum*
- *Ammophila arenaria*

Like the endemics of the neighboring bog flora and unlike the endemics of the vernal pools of the Great Valley, these species are probably relics. They reached California a long time ago and have since adapted themselves to only the most specialized habitats. It is perhaps more than a coincidence that *Agrostis blasdalei* and *A. pallens,* occupying isolated coastal situations, should be the only California representatives of their section of *Agrostis.* Other members of the section are found southward in the mountains of Mexico (*A. tolucensis* H.B.K.) and the mountains of Peru (*A. bromoides* Gr.). The species related to *Poa unilateralis* are also mountain inhabiting, but are found well represented in the California Sierra Nevada. Only *Elymus virescens,* most widespread of the three listed and the commonest (California north to Alaska), appears to be of recent origin, its nearest relative being the abundant *E. glaucus* of the Great Valley and the foothills.

Cooper (1936), in a comprehensive survey of the distribution and origin of the strand and dune flora of the Pacific Coast, concludes that *Poa confinis, P. douglasii, P. macrantha,* and the two dune species of *Elymus* (lumped by Cooper as *E. arenarius*) are, together with other associated species, of "subarctic or north temperate origin." The three *Poa* species—apparently closely related, for all fall into the section Pratenses—are related to *P. eminens* Presl of coastal northeastern Asia and arctic America and *P. labradorica* Steud., of the coast of Labrador. *Elymus* was first considered circumboreal in distribution, the North American material often being separated as var. *villosus* (St. John, 1915), but the latest taxonomic thought not only separates the New World material (as *E. mollis*) from the Old World, *E. arenarius,* but further distinguishes on the Pacific Coast *E. vancouverensis* Vasey. Although the origins of *Agrostis pallens* and *Agropyron arenicola* are more obscure, they belong to genera of principally north-temperate development, and they offer no evidence against the general picture of a migration southward of the grass species inhabiting the California sand dunes. Further evidence for the northern origin of the grasses in the dune flora is seen in the near absence of grasses on the strand formation of the southern California coast (McKenny, 1901).

There are only two introduced perennials on the dunes, *Ammophila arenaria* and *Agropyron junceum,* both from seashore sands and dunes of Europe, the former well established over a broader range than any of the native species, the second established only locally.

Salt-Marsh Grasses.—The ten species found in salt marshes are:

Native (all perennials)
- *Distichlis spicata* var. *stolonifera*
- *Hordeum nodosum* var. *boreale*
- *Monanthochloe littoralis*
- *Puccinellia nutkaensis*
- *Spartina foliosa*

Introduced
- *Agrostis palustris*
- *Lepturus cylindricus*
- *Lolium strictum*
- *Lolium subulatum*
- *Pholiurus incurvus*

Only the five introduced species, all from the salt marshes of Europe, have a similar background for their appearance in the California flora. It is striking that all but *Agrostis palustris* should be annuals, whereas no native annual grasses appear in the salt marshes. The general poverty of the salt-marsh floras probably attests to their recent formation, which is further evidenced by comparatively recent radical changes in the elevation of the California land mass.

Distichlis spicata is of wide occurrence throughout the Americas (Beetle, 1943*a*). Variety *stolonifera* intergrades with the inland variety *stricta,* and these may be presumed to be closely related. Variety *stolonifera* has neither become completely adapted to the salt-marsh habitat nor lost its connection with the inland flora. This situation is similar to that found in the introduced *Agrostis palustris,* whose affinities with the common pasture grass, *A. alba* (red top), are marked.

The dioeciousness of *Monanthochloe littoralis* is not an indication of relationship with *Distichlis,* in spite of the rare occurrence of the condition in the Festuceae. *Monanthochloe* is a small genus (probably only two species) ranging across the North American subtropics, from whence it came to California.

In contrast is the probable origin of *Puccinellia grandis,* which ranges north to Alaska; there its range coincides with that of at least twelve other related species (Swallen, 1944).

Finally, *Spartina foliosa* is similar to and probably related to *S. alterniflora* of the Atlantic Coast of North America; its method of reaching the California coast is in doubt.

Grasses of Bogs.—*Agrostis longiligula* and *Calamagrostis bolanderi* are the only two grasses within this habitat which need special mention. The true coastal bogs, strongly peaty and containing peat moss, have a flora independent of that of the recent bogs of both the Sierra Nevada and the Coast Ranges, whose species are essentially the same as those of the pine woods which surround them. It is likely that intensive search would reveal more bogs in the coastal areas than are now known botanically. At present, knowledge of them comes for the most part from Mendocino County where the largest and most southerly (Rigg, 1933) of the bogs occurs.

Unlike the endemic species of the Great Valley to be considered of recent derivation from typically California genera, *Agrostis longiligula* and *Calamagrostis bolanderi* are representatives of cosmopolitan genera and are possibly relics. Bog soceties are more common northward in North America and are closely related to similar societies in Eurasia (Transeau, 1903). According to Transeau:

> Present bog habitats are continuations of similar habitats which existed in early post-glacial times, when tundra conditions and tundra vegetation were dominant . . . [and] the temperature phenomena of undrained depressions, containing deposits of peat are favorable to the preservation of these types [that is, relics].

The more recently formed bogs tend to have a characteristic flora consisting of *Calamagrostis bolanderi, C. breweri, C. inexpansa, Glyceria pauciflora,* and *Deschampsia caespitosa.*

OTHER CALIFORNIA GRASSES

In addition, the grass family is represented in California by the bamboos, cereals, a few lawn grasses (*Opizia stolonifera, Axonopus compressus, Stenotaphrum secundatum,* and *Eremochloa ophiuroides*), and a few ornamentals (for example, *Cortaderia selloana* and *Ampelodesmos mauritanicus*), which are usually found only under cultivation. A partial list of introduced grasses and their histories is given by Robbins (1940); for additions see Beetle (1943, *b*).

CONCLUSION

Some of the foregoing material is available in floras of California and all of it could be. The application of this enlarged focus however, prepares the way for a new viewpoint in conservation work. The practical application of the present study in the field of agronomy centers around efforts to improve rangeland, a natural resource no less important than forest or wildlife, which has been found to require a program of planned management if its value is to be maintained undiminished (Jones and Love, 1945). The natural areas defined by the known ranges of grass species are the areas within which any given program of management will be practical.

In the search for better range grasses, the regions from which introductions might be presumed to be successful could be chosen on the evidence of: (1) incidence of successful introductions in the past; and (2) failure of unsuccessful introductions in the past, regardless of whether they have been brought in by nature before man or subsequently either purposely or accidentally by man. This is the type of evidence that should control the choice of native range plants for selection improvement or the choice of species and areas in reseeding programs.

In the larger and more general ecological picture, the evidence given by the detailed study of the grass family may now be correlated with the known facts concerning the migration of whole floras. Then greater confidence may be placed in the indicator value of grasses in studies of plant succession and range management. In addition, herein is basic evidence bearing on the question of grassland as a climax or subclimax.

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SOME PROBLEMS IN THE USE OF ARTIFICIAL LIGHT IN CROP PROTECTION[1]

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INTRODUCTION

WHEN THE AUTHOR first began to investigate the light reactions of blowfly larvae on the sandy beaches of Lake Erie, during the summers of 1903–1906, little did he suspect that he would be investigating the light reactions of the codling moth, *Carpocapsa pomonella* (Linn.), in apple orchards in California twenty-five years later. As the laboratory and field notes of the early work in Ohio are examined, two significant points that have been stressed throughout the years may be noted: (1) importance of knowing field habits, as then stated, "... behavior ... under natural conditions ... should be considered first"; this point was again stressed in 1909 (Herms, 1909); and (2) the importance of wave length and intensity. These early studies convinced the author of the powerful influence of light on the behavior of insects (and of other arthropods) (Herms, 1907 *a,b*), and suggested the possibility of devising ways and means for the use of artificial light in the control of insects for man's benefit in crop protection (Herms and Ellsworth, 1934). In spite of many interruptions and some opposition by old-line entomologists, as well as by other interests, some progress of economic importance has been made. As an aid to future work, examination of some of the problems encountered during the course of these investigations seems appropriate.

Much of the earlier work was concerned with laboratory studies (largely unpublished) of the color reactions of various species of insects of economic importance, such as the codling moth. These investigations indicated that insects have color preferences, and that such preferences differ markedly for the various species under consideration. Furthermore, the investigations seem to justify the belief that through the use of appropriate monochromats of sufficient light intensity, certain species of harmful insects could be lured to destruction with the exclusion of harmless and beneficial forms. Experimental evidence points to the fact that certain species of insects—particularly crepuscular forms—become active on exposure to low intensities of light and, by an increase in light intensity, might be deterred from performing their egg-laying function; hence the field tests made on the codling moth.

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LIGHT AS A DETERRENT

The first large-scale field tests in 1928 (Herms, 1929) were designed to test the deterrent effect of light on codling moths, as was also the second, a more elaborate test, made in the same orchard during the summer of 1929 (Herms, 1932). Under favorable conditions, codling moths deposit eggs freely within a range of light intensity from 0.3 to 90.0 foot-candles, and with maximum activity between 25 and 52 foot-candles. It therefore seemed worth while to test the efficacy of illuminating an orchard with artificial light that gave an intensity higher than optimum. In this way, it was thought the moths might be kept out of such light-protected plots and thus might be prevented from laying eggs within that area.

During the 1929 test, from April 24 to October 4, eighteen 500-watt Mazda lamps were suspended over the tops of the trees and were lighted from 2 to 4 hours daily during the sunset hours. Other factors being favorable, egg deposition takes place under natural conditions when the temperature during the sunset hours ranges from 60° to 69° F. At 55° to 58°, egg laying usually ceases. The measure of results at the end of the tests was the percentage of wormy apples; 217,975 apples, including thinnings and windfalls, from 51 trees, were carefully examined. The worminess was 49.7 per cent in a plot wholly illuminated and unsprayed, 71.3 per cent in an unsprayed, nonilluminated plot, and 7.4 per cent in a nonilluminated, properly sprayed plot. The reduction in worminess by only about 30 per cent, through the use of high-intensity light, was therefore of no great economic value. An examination of data shows an inability to maintain an adequately high degree of illumination throughout the test area (the range was from 11 to 112 foot-candles); certainly moths that entered the so-called protected plot did deposit eggs. As a matter of fact, a codling moth was seen to ovulate in a light intensity of 1,298 foot-candles. Borden (1931) records codling moths in flight at 1,928 foot-candles. Those female moths which were deterred from entering the highly illuminated area almost certainly went elsewhere to deposit their eggs. In that part of the orchard where artificial illumination kept the light intensity for the entire evening at from 0.3 to 0.4 foot-candles, there was a very high degree of worminess. To maintain a sufficiently high light intensity uniformly throughout the orchard during the critical egg-laying period appears to be exceedingly difficult and probably well beyond the margin of profit.

USE OF LIGHT TRAPS

Next studied was the possible use of light traps in codling-moth control. Some experience had already been gained (Herms, 1937) with field tests in light trapping other species, such as the Clear Lake gnat. Laboratory studies indicated a degree of attraction to certain colors; that is, when codling moths were exposed to light of several colors, but of approximately equal intensity, the response was to a pale blue with a wave length of nearly 4,350 Å. Two problems in particular were confronted in testing the use of light traps with colored light as a lure: (1) No suitable devices were commercially available to kill the moths lured to the light. (2) Appropriate and economical monochromatic light sources were not readily available. With the aid of Dr. L. T.

Jones, both problems were solved by the development of the needed devices: insect electrocutors and spiral gas discharge tubes which produce a large area of color (see fig. 1).

LIGHT TRAPS FOR CODLING-MOTH CONTROL

With the equipment described, the investigators went into the field during the summer of 1933. The test period extended from May 12 to October 16, with lights on from 5 p.m. to 5 a.m. A plot of 36 apple trees was used, and at the end of the experiment a fair sampling—that is, 19,220 apples—was made for worminess. Unsprayed trees under the protection of traps with pale-blue light (with a predominant wave length of about 4,350 Å—found to be most attractive in laboratory experimentation) gave a worminess of 50.3 per cent. This protection was about the same as that given by intense (repellent) white flood light during 1929—49.7 per cent. Unsprayed trees under protection of traps with red light (with a predominant wave length of about 6,800 Å) showed a worminess of 77.3 per cent. Unsprayed trees under protection of traps with white light (frosted 60-watt Mazda lamp) showed a worminess of 74.2 per cent—somewhat wormier than apples from a check plot, neither sprayed nor protected by traps, showing a 73.6 per cent worminess. Apples from trees having had only a routine arsenical spray treatment showed a worminess of 40.1 per cent (probably a defective spray program). To ascertain the number of codling moths killed by insect electrocutors at certain stations, catches were made and counted. Four traps with pale-blue light, which captured 2,930 codling moths, showed a sex ratio of 4 females to 5 males; 1 trap with 60-watt white light trapped 132 moths, with a sex ratio of 1 female to 3 males; and 1 trap with a 60-watt red light took 271 moths, with a sex ratio of 1 female to 2 males (Herms and Ellsworth, 1934). Although insect electrocutors with pale-blue light gave promising results, the use of this method as a substitute for an adequate spray program is not recommended for codling-moth control in apples.

EXPERIENCE GAINED FROM FIELD TESTS

The experience gained in this investigation has shown the complexity of any large-scale field project studying the use of light in codling-moth control. The need of thorough knowledge of the natural behavior of the insects involves the problem of employing adequate and well-trained assistants who will live day and night, over an extended period, in the native habitat of the insects. An essential problem in work of the type under consideration is, therefore, adequate financial support, which enables the employment of competent scientific assistance and the purchase of suitable equipment. Had it not been for the generous financial support of the California Committee on the Relation of Electricity to Agriculture, the field tests described in this paper, as well as many other tests, would have been impossible; this remarkable agency gave financial support to the investigation for more than ten years. Such investigations also require the generous coöperation of growers—a matter of no small importance.

Serious problems in the use of insect electrocutors in apple and pear orchards were encountered. One was the screening effect of dense foliage; the

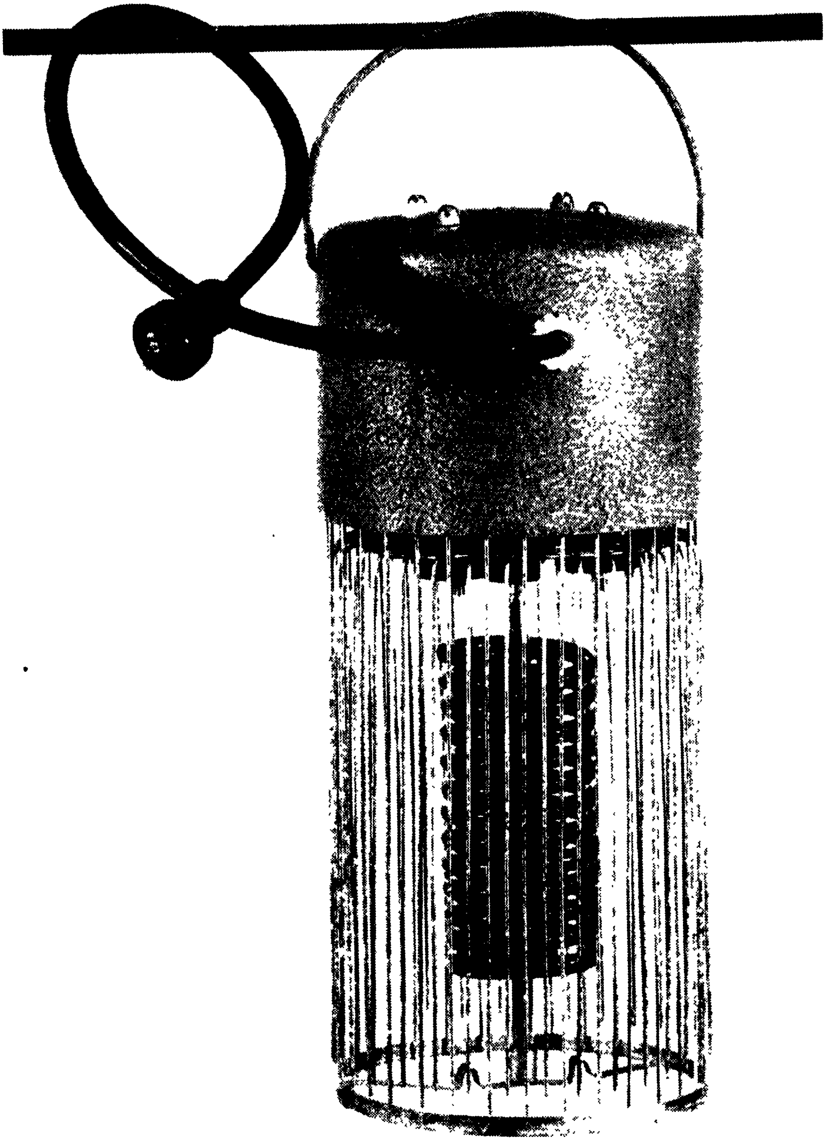

Fig. 1.—Insect electrocuting light trap used in experimental work.

height of the trees made necessary the use of poles and wiring to place the electrocutors sufficiently high. This problem led to even more elaborate field tests in vineyards, and in artichoke and tomato fields where the crop to be protected was not so tall and was more uniform. Here other problems, such as interference of wiring with cultivation and harvesting, were confronted. Throughout the investigation, safety was a factor of importance.

In the investigation of the use of light traps in crop protection, certain public-health and economic problems constantly have been kept in mind. These are the problems arising from the use of poisonous chemical sprays and dusts which leave a residue on fruits and vegetables and have a cumulative effect on soils. That first cost and operating expenses of devices used in crop protection must be kept at a minimum, commensurate with the margin of profit, is, of course, understood. The production of such equipment is a problem in itself.

LIGHT TRAPS FOR GRAPE LEAFHOPPER CONTROL

As was done in the experiment with the codling moth, tests were made in the laboratory to determine the color preference of the grape leafhopper, *Erythroneura comes* (Say). At the time, this insect was causing a heavy loss to vineyardists in the major grape areas of California. The grape leafhopper is strongly positive to light, and laboratory tests indicated a marked response to clear blue (somewhat lighter than pale blue). During the course of the field tests, begun in 1934 and covering a five-year period, 17 separate installations were studied; these represented a total of 156 traps in plots located over a wide area in the San Joaquin Valley.

Tests made for relative effectiveness of (all 60 watt) (1) clear blue (a new light developed for this purpose and found by laboratory tests to give the greatest degree of response), (2) pale blue (previously found to be effective for codling moth), (3) dark blue, (4) red, and (5) white incandescent lamp, showed that the clear blue attracted greater numbers of leafhoppers in the field than any other light; the clear blue attracted more than twice as many hoppers as did the white incandescent lamp, and the dark blue attracted about the same number as the red. However, about four times as many females were taken by the trap with the dark-blue light as by the trap with the white, and ten times as many as by the trap with the red light. The sex ratio for the red light was 10 males to 1 female. Later tests indicated that the clear blue attracted not only the largest number of hoppers but also the highest percentage of females.

Population studies indicated that one insect electrocutor per acre killed a sufficient number of leafhoppers daily to afford protection. Therefore, the practice of using one trap with clear-blue light, per acre, was adopted for practical purposes. Tests made with lights (Herms and Ellsworth, 1935) of the same color, but with increased intensities, proved interesting and of practical value in that an increase in intensity from 60 watts to 150 watts increased the number of leafhoppers from 399 to 844 per trap per night—more than twice the number. Also, during a period from August 27 to October 23, 1934, a trap with a 60-watt dark-blue light took a total of 103,000 hoppers, an average of 1,758 per night, while a trap in the same general area with a 120-watt pale-blue light took a total of 264,000, or an average of 4,551 leafhoppers per night—better than two and a half times as many. Had the investigators used the clear-blue lights, referred to earlier, for this test, undoubtedly the number of leafhoppers captured would have been increased even more.

That the proper location of traps, with reference to the vineyard as a whole, is important is indicated by the fact that vineyards in which light traps were used showed heavy foci of Pierce's disease of grapevines in the immediate vicinity of light traps. In fact, it was this observation that caused suspicion to rest on leafhoppers as vectors of this disease. These traps attract vast numbers of leafhoppers of many species. The grape leafhoppers are small and fly readily into the insect electrocutors; other leafhoppers (sharpshooters) are heavy bodied and are freely attracted to the light, but they collect in great numbers in a rough circle below the light traps. During 1941 conclusive proof was shown that Pierce's disease was transmitted by two species of leafhoppers, namely, *Draeculacephala minerva* Ball and *Carneocephala fulgida* Nott. (Hewitt, *et al.*, 1942). Both species feed on alfalfa and wild marsh grasses; the infection they transmit to alfalfa causes alfalfa dwarf. Although the evidence is only circumstantial, the belief exists that the lights attract the hoppers from adjacent infected alfalfa fields into the vineyards and thus cause the foci of Pierce's disease in the vicinity of the traps. If this evidence

TABLE 1

INSECTS TAKEN DURING TWO NIGHTS (AUGUST 23 AND 24, 1938) BY LIGHT IN A VINEYARD

(Traps with 60-watt pale-blue lights, at different elevations)

Insects*	Elevation		
	7 ft.	12 ft.	17 ft.
Coleoptera, total	*5,692*	*5,044*	*5,089*
Carabidae	987	550	813
Dytiscidae	480	1,051	321
Hydrophilidae	136	226	210
Chrysomelidae	1,231	778	870
Coccinellidae	1	22	0
Curculionidae	169	447	576
Hemiptera, total	*298*	*685*	*861*
Miridae	249	227	740
Lygaeidae	25	14	31
Reduviidae	2	28	15
Homoptera, total	*104*	*568*	*890*
Cicadellidae	104	568	888
Lepidoptera, total	*567*	*331*	*205*
Noctuidae	49	29	25
Microlepidoptera	508	262	165
Diptera, total	Collection lost	*622*	*4,554*
Chironomidae		164	2,097
Sciaridae		211	2,001
Muscoidea		179	224
Hymenoptera, total	*12*	*3*	*3*
Ichneumonidae	5	2	0
Trichoptera	*19*	*17*	*4*
Total, all insects	*6,692*	*6,448**	*7,052*†

* Totals for orders also include insects not shown by families.
† Exclusive of Diptera.

is true, it presents a problem of such proportions that further investigation is required. The great peril to the grape industry presented by Pierce's disease renders undesirable what otherwise might have been a practical control of the grape leafhopper in certain areas.

USE OF LIGHT TRAPS AGAINST ARTICHOKE PLUME MOTHS

Another important crop which was believed to lend itself to a study of light traps in protection against a serious pest—the plume moth—was the artichoke. Laboratory tests showed that the artichoke plume moth, *Platyptilia carduidactyla* (Riley) was strongly phototactic, was a night flier, and responded most favorably to a dark-blue light. Extensive field tests were conducted during the period from 1936 to 1938; although encouraging results were obtained, indications pointed to the fact that light traps, when used as a sole means of control, did not offer adequate protection. However, during the period of July, 1937, to December, 1938, further tests were made in an 8-acre field with one 60-watt dark-blue light trap used per acre. At sunset and at sunrise the traps were turned on and off automatically, unless the temperature dropped below 50° F, under which conditions the thermostat opened the switch and the lights went off. The number of plume moths captured for the 18-month period was 2,032, an average of 3.85 moths a day for 8 traps. The sex ratio was 1 female to 5.5 males. A 60-watt white incandescent lamp was tried but very few female moths were attracted. A general summary is given by Lange (1941) as follows:

> Important factors which were found to influence the capture of moths at light traps were, first, the height of the traps, second, night temperatures, and third, the intensity of wind currents. An experiment in which light traps were placed at different heights from the ground, demonstrated that most moths were taken about the level of the artichoke plants, usually from 3 to 5 feet, with a few taken at 8 feet, but none at 12 feet. The moths were found to be capable of reaching considerable heights in the air when aided by wind currents, but were not able to direct their flight sufficiently to hit a light trap placed at 12 feet in the air. Night temperature was found to be an important factor in that often a difference of only 2° F determined the difference between moths flying or not flying. For purposes of light-trap operation, 50° was determined as the minimum flight temperature, although flight was observed down to 46°. Wind currents played an important part in the capture of plume moths at light traps in that once the moths were carried beyond the traps they could not fly back. During the summer it was found that most flight begins at dusk and continues until midnight, the time depending primarily upon temperature.

USE OF LIGHT TRAPS IN CORN-EARWORM CONTROL ON TOMATOES

To test the use of light traps in the control of the corn earworm, or tomato fruitworm, *Heliothis armigera* (Hbn.), in tomatoes, three plots were set up for a period of about 3 months in four widely separated tomato-growing sections of California. These plots consisted of 20 acres of tomatoes with 1 trap (60-watt pale-blue light) to 2 acres; and two plots of 6 acres each with 1 trap (60-watt pale-blue light) per acre.

The number of moths taken per trap per night reached a peak about September 25, when the average for the 60-watt pale-blue light was 29, with a sex ratio of 1 female to 1 male. The average per trap per night for the entire season was slightly over 3, with a sex ratio of about 4 females to 6 males.

Four 60-watt light traps located in the same area and operated from July 27 to August 27 (1936) gave the following results:[3]

Source of light	Corn-earworm moths	Sex ratio
One 60-watt pale-blue light	75	3 females to 7 males
One 60-watt red light	29	2.5 females to 7.5 males
One 60-watt white Mazda lamp . . .	74	2 females to 8 males
One 60-watt daylight Mazda lamp . . .	43	3.7 females to 6.3 males

In the artichoke investigations conducted at Moss Beach from August 1 to August 30, 1936, Lange (1941) used 4 traps, each containing a 60-watt dark-blue light. He captured an average of about 150 corn-earworm moths per trap per night, for the 30-day period, with a sex ratio of 1 female to 1.7 males.

HEIGHT OF TRAPS

The height of the light traps has already been mentioned. To ascertain the height at which codling moths fly, traps were placed in an apple orchard. One trap was placed about 2 feet above the tops of the trees—about 21 feet high—the other at an elevation of 35 feet. Both traps had 60-watt pale-blue lights. During the month of July the trap placed at a height of 21 feet captured 399 codling moths (53 per cent females) and the trap placed at an elevation of 35 feet captured only 29 moths (51 per cent females) (see footnote 3).

Another test was made by placing 3 traps, each with a 60-watt pale-blue light, over a vineyard at heights of 7 feet, 12 feet, and 17 feet, respectively. A tabulation of the catch for the 2 nights (counting only Coleoptera, Hemiptera, Homoptera, Lepidoptera, Hymenoptera, and Trichoptera, given in table 1) shows little difference in the total number of insects taken at the traps. If the Diptera, eliminated because of lost records, were included in the catch for the 17-foot elevation, this elevation would show a distinct advantage with a very large collection of Chironomidae and Sciaridae. Both the Hemiptera and Homoptera showed increased catches at increased elevation; but the Lepidoptera showed a decrease at increased elevation, particularly noticeable in the Microlepidoptera. This decrease was also very marked in the Chrysomelidae (Coleoptera).

LIGHT TRAPPING OF INSECTS IN PACKING-HOUSE AND FIG DRYING YARD

In an experiment conducted by Ellsworth (1939) (see footnote 3) at a packing-house, during the period March 29 to July 7, 1938, a total of 6,920 codling moths was electrocuted through the use of 5 insect electrocutors. The lights were rotated counterclockwise at two-week intervals to equalize any advantage in position. The results were as follows:

Source of light	Codling moths trapped	Per cent females
One 120-watt pale-blue light	3,743	46.2
One 60-watt pale-blue light	1,847	48.1
One 60-watt Mazda lamp	902	44.3
One 15-watt dark-blue light	428	51.5

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[3] Ellsworth, J. K. A critical and experimental study of the use of light, particularly monochromatic light, in the control of insects. Thesis for the degree of Doctor of Philosophy, University of California, 1939. (Typewritten.) Copy on file in the University of California Library, Berkeley, Calif.

Two insect electrocutors, one with dark-blue and the other with pale-blue light, were operated for 4 successive nights (October 11 to October 15, 1933) in a fig drying yard at Fresno (California) where figs are dried on stacked trays. Moths were quite numerous in this yard. In the summarized results which follow[4] apparently all of the *Ephestia* sp. were *E. figulilella* Greg. Of the 1,578 captured by the dark-blue light, 51.9 per cent were females, and of the 739 captured by the pale-blue light, 53.4 per cent were females. Many of these females were gravid.

	Dark-blue light	Pale-blue light
Noctuidae	523	340
Ephestia sp.	1,578	739
Moodna nigrella (Hulst)	17	10
Nemeritis canescens (Grav.)	2	1
Microbracon sp.	2	2
Pyralis farinalis Linn.	0	1

CLIMATIC FACTORS THAT MILITATE AGAINST USE OF LIGHT TRAPS

Many climatic factors militate against the use of light traps as a general means of insect control—temperature, humidity, and also wind. Lawson (1930) collected leafhoppers for species studies by using a light trap, equipped with a 200-watt Mazda bulb, suspended 8 feet above the ground. In discussing the results of collecting leafhoppers for two seasons in this manner, Lawson comments as follows:

> It is perfectly clear that cold weather, severe rains, and high winds keep leafhoppers close to the ground or in their diurnal habitats. It is not so easy, however, to state the conditions under which they swarm to lights in unusual numbers... but favorable weather conditions are absolutely necessary if they are to fly to lights in large numbers.... If a large number of leafhoppers are present they will swarm to lights when there is little wind, and when there is a favorable combination of high temperature and high humidity.

The great irregularity with which leafhoppers appear at lights is shown in Lawson's table of catches for *Draeculacephala mollipes* (Say) and *Euscelis obscurinervis* (Stål.). The total catch of the former for August was 59,713 and the total catch of the latter for September was 112,998. For the former, there were only 2 nights when the catch was between 20,000 and 23,000; 1 night, 8,409; 2 nights between 1,500 and 2,000; and all other nights under 900. In the case of *E. obscurinervis* there was a catch of 70,225 during 1 night and the next nearest catches for 2 nights were between 10,000 and about 11,000. On most other nights the catches were less than 800; and on several nights none was caught.

Every entomologist interested not only in light traps but also in the behavior of insects generally should read and carefully study the masterful contribution by Williams (1940). Williams points out that:

> It is impossible to study insects in the field without realizing to what a large extent their activity and abundance are determined by the effect of the various climatic factors of the environment. It is, however, a big step from the realization that some of the factors have a large and some a smaller effect, to a proper analysis which will show on some scale of measurement exactly how much effect is produced in the fauna by a particular extent of change in one particular factor of the environment.

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[4] Information furnished through the courtesy of Perez Simmons, Dried Fruit Insect Laboratory, U.S. Bureau of Entomology and Plant Quarantine.

Williams groups the weather conditions under six headings: (1) temperature; (2) radiation (light); (3) moisture; (4) wind; (5) atmospheric pressure; and (6) thunder and lightning. He points out that temperature is probably the most important single factor in the climate of the cool temperate zone in a locality such as Rothamsted (England).

Bordon (1931), referring to the codling moth in particular, states:

> [Its] flight is governed by temperature, humidity, light intensity, and air movements. Just which of these are the most important is difficult to state; however, light intensity, temperature, and air movements seem to play a very important role. In the Sebastopol district (California) which has a relatively low humidity as compared with the Watsonville district (California) a rise in temperature gives a decided drop in humidity. The appearance of the fog from the coast in the early evening very quickly drops the temperature and stops moth flight. The maximum temperature at which flight has been observed to start is 78° F and the minimum temperature for this district is 54° F at Sebastopol, while in the Watsonville district 55° F seems to be the average minimum. Too high temperatures in the early part of the evening have shown a decided check on moth flight during the months of June and July.

THE INTENSITY FACTOR

Numerous experiments were made (see Ellsworth citation, footnote 3, p. 364) with the grape leafhopper, *Erythroneura comes* (Say), to ascertain the effect of different intensities and color. As pointed out above, when the intensity was increased from a 60-watt incandescent lamp to a 150-watt lamp the number of leafhoppers per trap per night was increased from 399 (38 per cent females) to 844 (40 per cent females); and when the intensity was increased from 150 watts to 300 watts the number of leafhoppers was increased from 590 to 1,445 (49 per cent females) per trap per night. It was also pointed out that during the period from August 27 to October 23 a trap with the 60-watt dark-blue color took a total of 103,000 leafhoppers, or an average of 1,758 leafhoppers per night, while the trap with 120-watt pale-blue light took a total of 264,000, or an average of 4,551 leafhoppers per night. A doubling of the wattage and a change in color resulted in better than a 2½-fold increase in the number of hoppers.

The following quotation from the author's bulletin on the Clear Lake gnat (Herms, 1937) is evidence that the intensity factor is important.

> The next step was to determine whether the number of gnats attracted to lights was proportional to the intensity. A battery of lights (25-, 40-, 100-, 150-, 300-, 500-, 1,000-, and 1,500-watt incandescent lamps) was set up along the shore. It was found that the number of gnats captured per second increased rapidly in lamps of from 25 to 300 watts and decreased in number when 1,000 watts was reached; this indicates a point of deterrence. There are 360 gnats in 100 milligrams or 1,650,000 gnats in a pound. Over a 15-minute period the 300-watt lamp captured (approximately, by weight) 1,650 gnats per second. The 1,500-watt lamp attracted approximately the same number of gnats as the 200-watt lamp. Up to the point of deterrence the number of gnats attracted to a light is directly proportional to the increase in the intensity of that light. It was concluded that the number of gnats collected by any similar device depends upon the intensity of the light and the rate at which they are removed from the apparatus.

THE WAVE-LENGTH FACTOR

Bertholf (1931*a*) points out three fundamental questions to be considered in the reactions of a given animal to light of different wave lengths: (1) the extent of the spectrum for this animal—the wave lengths of the shortest and longest radiations to which it responds; (2) the relative efficiency of the different wave lengths eliciting the same response; and (3) the extent to which the animal distinguishes the different wave lengths or the number of chromas that it distinguishes. For his experimental work, Bertholf used a spectroscope (monochromator) of the constant-deviation type, with which desired wave lengths could be obtained. In a later publication, Bertholf (1931*b*) states that for his study of the relative efficiency of the light from certain lines of the ultraviolet spectrum he used a mercury vapor lamp, of quartz, the light from which was passed through a spectroscope (monochromator) provided with lenses and prisms of quartz. He found that when the curve representing the distribution of stimulative efficiency for the ultraviolet spectrum is joined to that for the so-called visible spectrum, as he found in his previous investigation (Bertholf, 1931*a*), two maximums are shown for the honeybee: one is in the yellow-green, at a wave length of 553μ, which corresponds rather closely to that of man, and the other is in the ultraviolet at 365μ, which is about four and one-half times as great as the former.

In reporting results of tests made on various species of insects, Gross (1913) states:

> The relative stimulating efficiency of the rays of any part of the spectrum is independent of intensity, and is not the same for all animals nor for different ages of the same animal.... *Periplaneta americana* is responsive to blue, green and yellow, but is indifferent to red. It is positive to blue and negative to both green and red.

Gross used Nernst glowers on a 220-volt circuit as the light source and obtained the colors by cutting down the spectrum by means of diaphragms of cardboard; the finer adjustments of intensity were accomplished by regulating the size of the diaphragms until light gave the same reading on a radiomicrometer.

To determine the color reactions of insects in the tests here reported, various cabinets and devices were used. These are described in detail by Ellsworth (see footnote 3, p. 364). Eastman Wratten filters were among the chromats used. The transmission spectra for the various filters were photographed. The intensity of the lights was measured and equalized by the use of a linear thermopile, galvanometer, and potentiometer. Evidence gained in the laboratory indicated a degree of response to color that differed (often but slightly) for the several species tested. Lack of equipment to produce monochromatic light of various wave lengths of high intensity, even in the laboratory, accounts for the extremely crude color designations used in this paper. For field use, the lack of monochromatic light of high intensity was even more of a problem. Table 2 shows numbers of insects taken in a vineyard, by electrocutors with different colors of light, during the night of August 22, 1938. Pale blue has first place; black (ultraviolet) has second; dark blue, third; yellow-green, fourth; and red, least.

SEX RATIO OF LIGHT-TRAPPED INSECTS

Although not substantiated by a sufficient body of proof, the statement is generally made that white light should not be used in crop-protection operations against insects, because many beneficial forms are thus destroyed. Certainly white light does attract a large variety of night-flying phototactic insects, among them beneficial species—a matter which will be discussed later. However, one definite objection to the use of white light is that it attracts a preponderance of males and that such females as are attracted show, by ovarian examination, that a large percentage have already deposited their eggs. In considering sex ratio of insects taken at light traps, one should have a knowledge of the normal sex ratio under field conditions. Thus Turner (1918) states that the codling moth occurs in nature in a ratio of 5.5 (55.6 per cent) males to 4.5 (44.4 per cent) females. Since the sex ratio is subject to a variety of factors, one may reasonably assume that the ratio may differ for various localities; however, the ratio of 5 males to 4 females in the codling moth may be accepted with reservations. By using a 300-candle-power arc lamp among the moths, Turner (1918) collected 1,289 codling moths; of these 44.7 per cent were males and 55.3 per cent were females, a difference which indicates that more females than males are attracted to light. However, the sex ratio of all species of moths taken by Turner in his test was 71.5 per cent males to 28.5 per cent females, 58 per cent of which were gravid. In the tests of Herms and Ellsworth (1934) 2,930 codling moths attracted to a 60-watt pale-blue light showed a sex ratio of about 4.5 males to 5.5 females, the same sex ratio that Turner showed for white light.

TABLE 2

INSECTS COLLECTED IN A VINEYARD BY TRAPS WITH LIGHTS OF DIFFERENT COLORS BUT OF THE SAME WATTAGE (60), AUGUST 22, 1938

Insects	Light traps				
	Pale-blue light	Yellow-green light	Red light	Dark-blue light	Black light
Coleoptera, total	*2,480*	*305*	*61*	*1,120*	*1,348*
Chrysomelidae	117	7	0	21	19
Carabidae	321	65	16	186	187
Dytiscidae	359	25	3	301	429
Hemiptera, total	*85*	*49*	*6*	*37*	*49*
Miridae	59	40	4	14	4
Lygaeidae	5	7	2	3	1
Homoptera, total	*262*	*135*	*66*	*67*	*89*
Cicadellidae	260	134	49	65	89
Lepidoptera, total	*94*	*98*	*6*	*120*	*145*
Noctuidae	14	2	0	4	15
Microlepidoptera	66	40	4	102	85
Diptera, total	*515*	*213*	*61*	*239*	*1,202*
Chironomidae	0	41	36	5	1,088
Sciaridae	308	0	0	120	0
Culicidae	98	33	13	29	21
Hymenoptera	*2*	*1*	*0*	*0*	*3*
Neuroptera, total	*0*	*0*	*1*	*0*	*0*
Chrysopidae	0	0	1	0	0
Total, all insects*	*3,438*	*801*	*201*	*1,583*	*2,836*

* Totals for orders also include insects not shown by families.

Tests with the artichoke plume moth reported by Herms and Ellsworth (1934) showed that few moths were attracted to a 60-watt incandescent lamp (white) with a sex ratio of 1 female to 8 males, while the sex ratio for 99 moths of the same species taken at a 60-watt dark-blue light was 3 females to 2 males. Lange's test (Lange, 1941) with the same color and wattage of light gave for 2,082 moths a sex ratio of 1 female to 5.5 males. Such extreme variations in results indicate an array of problems, largely climatological.

CONSERVATION OF BENEFICIAL INSECTS

In a two-day test on August 23 to 24, 1938, made over a vineyard with 22 traps, and using lights of various colors and intensities, a total of 110,225 insects was trapped and later segregated into orders and families. Of this total, taken at various traps, only 19 were Chrysopidae and 70 beneficial Hymenoptera. Much more research needs to be done before a conclusive answer can be given as to whether or not light traps constitute a menace to beneficial insects. Surely the percentage taken under the conditions stated above is extremely small, namely, about 0.008 per cent, hardly sufficient evidence to condemn the use of properly controlled light trapping in crop protection operations (Patch, 1938). A review of the investigations of the use of light in crop protection would soon convince any unprejudiced entomologist (or conservationist) that the investigators were opposed to the indiscriminate use of light traps and that they had as their objective the discovery of a monochromatic light specific to a given pest, to the exclusion (or the saving) of other insects not involved in the damage (Herms, 1934).

SAFETY FACTOR IN THE USE OF ELECTROCUTING LIGHT TRAPS

The placing of light traps high above the ground for use against orchard insects offers little danger; but when they are used in the protection of grapes, artichokes, and tomatoes, where they require elevations nearer to the ground, the safety factor in insect electrocutors must be taken into consideration. These traps cannot be economically protected by wire basket guards. Limiting the current to make them harmless to human beings and animals seems to be the simplest way of eliminating the hazard. Tavernetti and Ellsworth (1938) pointed out two problems that had to be solved in order to obtain the safety factor: (1) the determination of the current that is safe for human beings and animals; and (2) the determination of the current and voltage necessary for the efficient operation of the device. A third point, the current necessary to kill the various species of insects, must also be determined.

Experiments were conducted by Dalziel (1938) to determine the amount of current that can be voluntarily tolerated by humans. He reported on tests with 56 men between twenty and forty years of age, in which 60-cycle current was used. In these tests, 13.9 milliamperes was the average and 22 milliamperes the maximum tolerable current from hand to hand, and 12.6 milliamperes was the average and 18 milliamperes was the maximum current which could be tolerated, yet permit the person to release a conductor grasped by the hand. Dalziel also reported that the amount of tolerable current was dependent on the frequency but independent of the voltage.

Tavernetti and Ellsworth (1938) used potato tuber moths and green-bottle flies to determine the electrical requirements for efficient operation of the electrocuting devices in killing insects. At least three important factors must be taken into consideration: (1) voltage between grid bars; (2) average line current; and (3) spacing of grid bars. Tavernetti and Ellsworth found that up to about 15 milliamperes' current drain, the voltage needed for electrocution decreased fairly rapidly with an increase in current. Little was gained in going above 15 milliamperes and there is a minimum voltage below which a 75 per cent kill is not possible regardless of the current. Approximately 250 to 500 volts are required for each 1/32-inch spacing between grid bars to obtain a 75 per cent kill.

These authors point out that, in the practical use of electrocuting grids on insect electrocuting traps, various factors other than the killing of the insects must be considered in determining the electrical requirements: (1) The use of high voltage makes insulating more difficult and increases the corona loss (when about 5,000 volts were exceeded, the corona was both audible and visible in a dark room from the loose ends of the bars). (2) The line current can never be less than that needed for the light at proper intensity; the use of neon light (which is common where colored light is used) presents a problem in reducing the current; and, since the intensity of the light is important in attracting the insects, reduction in current reduces the intensity and thus cuts down the number of insects attracted. (3) Since the grid must be kept clear of dead insects, the grid-voltage transformer must be of sufficient size to supply enough current to burn the insects or to knock them off by arcing, when they stick on the grid.

During the summer of 1927, Herms and Burgess devised a suction-fan trap as a substitute for insect electrocutors in the control of the Clear Lake (California) gnat. The electrocuting devices quickly became clogged and inoperative until cleaned, hence the idea of a device in which there was installed a fan with a motor which sucked the insects down from the light (a 300-watt lamp) into a black muslin bag. With one of these devices (Herms and Burgess, 1928; and Herms, 1937), 2 pounds of gnats were trapped in 2 hours, or a total of 156 pounds during one summer. Among the difficulties encountered in the operation of these devices were: (1) clogging of the fan with gnats, a condition which required almost constant attention; and (2) many of the gnats remaining alive in the bag, which made their disposal difficult. Traps of this type are comparatively safe to operate.

DISCUSSION

Presented in this paper is a summarized account of certain experiments made with artificial light, particularly with insect electrocuting light traps under field conditions, in the control of insects. This exploratory work covers a period of more than twelve years of field operations with such crops as apples, pears, grapes, artichokes, and tomatoes. The following species of crop pests were used in the experiment: codling moth, *Carpocapsa pomonella;* grape leafhopper, *Erythroneura comes;* artichoke plume moth, *Platyptilia carduidactyla;* and the corn earworm or tomato fruitworm, *Heliothis armigera.* Laboratory experiments on a limited number of species indicate a possible specific color selection; and if light traps could be devised with monochromats appropriate to certain phototactic insect pests, a method of crop protection that would not destroy beneficial insects in appreciable numbers might be developed. Such a method of crop protection would avert the residues on fruits, berries, and vegetables which result from poisonous sprays and dusts. Deleterious effects on soil from accumulations of insecticidal sprays and dusts, such as arsenic, would also be avoided.

Much more refined experimental laboratory work must be done to prove conclusively the theory of color selection. Light traps for field operations must be provided with monochromats. Present available traps do not usually have light sources that produce monochromatic light of sufficient area and intensity. Much more correlative work must be done in the laboratory and in the field. Technical equipment and administrative coöperation are needed.

Experience gained in the field has impressed investigators with the complexity of any large-scale field project. A thorough knowledge of the natural behavior of the insect under all ecological conditions is essential in this type of investigation. A vast number of problems are encountered, and this paper attempts to evaluate such ones as climatological factors, position of traps, intensity of light, size of light source, and color (wave length). Each problem must be considered with adequate knowledge of the particular species under experimentation.

The measure of success in the use of artificial light, particularly the various types of light traps, in crop protection is commonly indicated by the numbers of insects destroyed. The grower is not much impressed with the numbers (though great) of insects destroyed by electrocuting light traps, for example, but he is very much interested in the degree of *worminess* and the *grade* of the fruit. While a measure of effectiveness is not indicated in terms of numbers of insects taken per trap per night, most of the reports used for Experiment Station purposes are in percentage of worminess; these reports are comparisons between plots receiving no chemical treatment and plots receiving standard applications of sprays or dusts. The percentages of worminess of apples, artichokes, and tomatoes show a fairly close inverse correlation with the numbers of particular pests destroyed by the electrocutors. With grapes, success was measured in terms of the grade of grapes harvested. With apples, in the same test plot under artificial white-light illumination during the flight period of the codling moth for two consecutive seasons, the worminess was about 50 per cent compared with a worminess of about 71 per cent in nonilluminated and nonsprayed test plots and a worminess of about 7 per cent in a nonilluminated, properly sprayed plot. Little can therefore be said in favor of using the so-called intense white light as a deterrent. The use of insect electrocuting light traps equipped with pale-blue light (about 4,350 Å) resulted in holding down the worminess of apples to about the same degree as did the repellent white light, namely about 50 per cent compared with about 75 per cent worminess in an unsprayed check plot without light traps. Tests made on tomatoes and artichokes were too limited in extent to derive conclusive evidence, although the fruits of both lend themselves well to a study of worminess. Engineering problems in setting up a system of wiring for light traps in cultivated fields differ materially from those used in orchards.

Economical as well as effective light-trap installations were used on a large scale in various parts of the San Joaquin Valley. These traps resulted in markedly higher yields and in high quality of grapes because of lessened damage by leafhoppers (Ellsworth, 1938). Unfortunately, because of the far-reaching power of photic attraction, leafhoppers infected with the virus of Pierce's disease of grapevines may be drawn into a vineyard from some distant point, with serious consequences. The use of electrocuting light traps with appropriate monochromats in grape-leafhopper control gives much promise were it not for the presence of this disease of the vine. The promising results achieved in the control of the Clear Lake gnat warrant further study of this problem as well.

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A PHYSIOLOGICAL STUDY OF BORON DEFICIENCY IN PLANTS[1]

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INTRODUCTION

PROBLEMS RELATING to the nutrition of plants have assumed an increasing importance since it is now evident that the so-called micronutrients play an important role not only in the physiology of the plants but often of animals which feed upon them. True, most soils contain these elements, but soils are known in which the amounts necessary for healthy plant growth fall below the critical level.

It is a matter of record that the systems of farming in California which expanded horticulture in the latter part of the nineteenth and early twentieth centuries were for a time highly successful, but eventually in certain districts the fruit and vegetable crops were affected with hitherto unknown maladies unrelated to attacks of plant-disease organisms or of insects. The new maladies were termed "dieback," "yellow leaf," "sour sap," "little-leaf," "mottle-leaf," "cork spot," "decline," and others, all of which connoted a general lack of vitality and fruitfulness. In an attempt to remedy the condition, soil amendments which included a wide range of materials were applied to orchards and fields, sometimes with success, but often contrariwise. When conventional methods of treatment failed to ameliorate the situation, it became apparent that new studies must be undertaken to ascertain the underlying causes of the unexplained crop failures.

The problems were investigated from various angles; often false starts were made, but eventually it was discovered that small amounts of the micronutrients could overcome the unthrifty condition of some of the trees. An extensive series of experiments has demonstrated that the application of small quantities of manganese, or iron, or boron, or zinc can often restore unhealthy plants to vigor. Usually the salts of these elements are applied directly to the tree, but in vegetable growing they are applied to the soil.

Many of the problems connected with this phase of plant nutrition are still unsolved. Additional investigations are therefore essential to obtain accurate

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information about the underlying causes of the abnormal behavior of plants whose nutrition is deficient in one or another micronutrient. But haphazard treatments and empirical methods have been displaced by scientific researches. There is no substitute for accurate information derived from controlled observations and controlled experiments.

The apparent lack of micronutrients in the soils in many parts of the world emphasizes the broad scope of the problems. Space forbids the briefest mention of the studies made by many workers on many plants in many lands which year by year have added to the store of information on the subject. The economic and scientific importance of the problem justifies researches on the effects of these hitherto unappreciated factors in plant growth.

The present paper deals with the effects of boron deficiency in the olive, celery, and sunflower, with brief consideration of some other plants. Clinical symptoms of the affected plants are described as well as possible to facilitate the diagnosis of the maladies ensuing from an inadequate boron supply or from adverse conditions which impair the power of the soil to furnish boron. The derangements in the conducting systems of the plants, as well as in the centers of synthesis of substances necessary for growth, are considered. Especial attention is devoted to cytochemical features of the leaf, of the primary meristem, of the phloem elements, and of other cellular structures.

The author expresses his gratitude to many colleagues for their helpful suggestions and criticisms during the course of the investigation.

OLIVE

Boron deficiency in the olive tree has been recently described by Scott, Thomas, and Thomas (1943),[3] who have studied the effects in bearing and young trees and have found that the symptoms are readily identifiable. During fall and winter some of the leaves of the current season's growth became pale green in the apical portions, whereas the basal part of the laminae retained the normal green color. Some leaves which had been chlorotic for several months developed bands of necrotic tissue in the distal regions. Affected trees, owing to the death of terminal buds and subsequent secondary growth from lateral buds, had a bushy appearance. In severe cases these secondary shoots may die in the same season. Branches often died at the end of the growing season, although they had shown good growth and very mild symptoms a short time previously. Haas (1945) reported data on the chemical aspects of the nutrition of the olive, especially on the translocation of boric salts into the leaves.

The first-mentioned investigators kindly placed at my disposal much of the material used in the present study. Small trees grown in soil deficient in its boron-supplying power produced dwarfed shoots and leaves (fig. 1) unless boric acid was added to the soil. Characteristically the terminal bud on the main shoot of the young tree died at the end of the first season's growth. Subsequent shoot growth developed from upper lateral buds, giving the tree a Y shape, or from basal buds which had been previously inhibited by the dominance of the main axis.

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[3] See "Literature Cited" for complete data on citations, which are referred to in the text by author and date of publication.

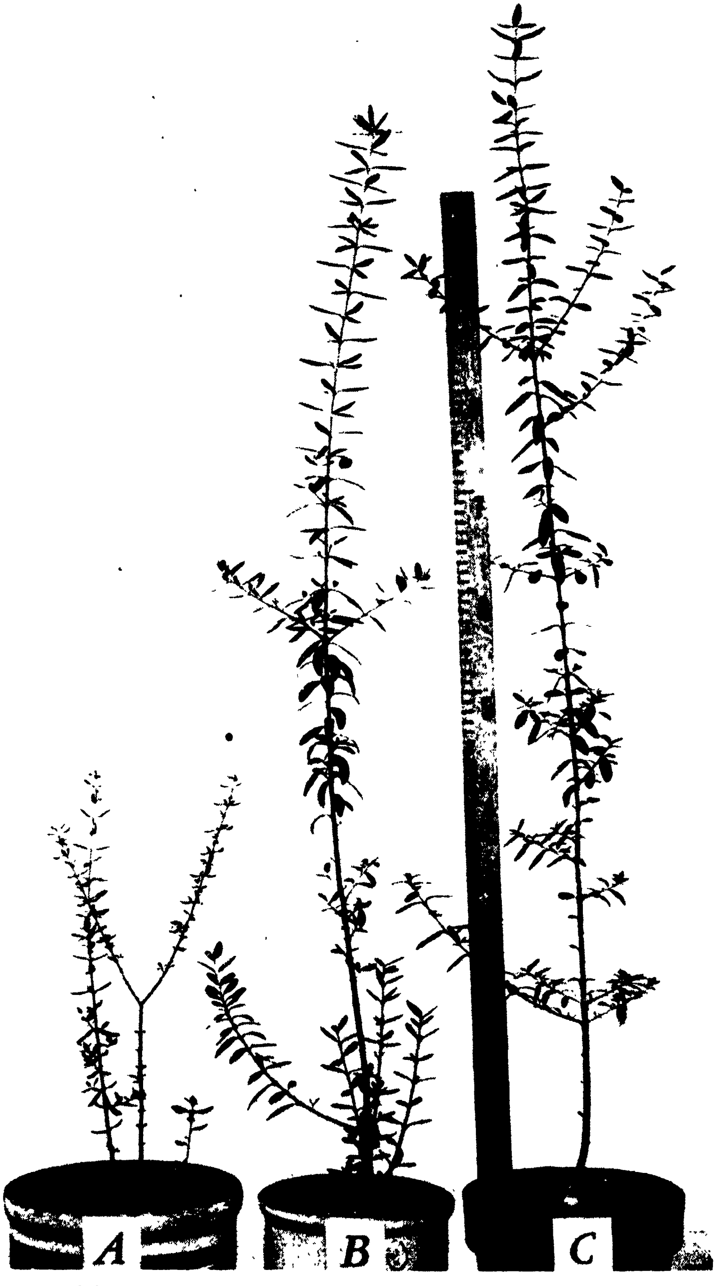


Fig. 1.—Olive trees grown in soil which was known to have a low boron-supplying power. Jar *A*, no boron added; *B*, 0.5 gram boron added; *C*, 0.12 gram boron added. (Photographed May 18, 1945, by courtesy of the Division of Plant Pathology.)

The growth of the apical meristem and the development of the various rudiments which arise from it command attention in any physiological study. Not only is boron necessary for the growth but also for the life of the terminal bud; yet, after the death of the terminal bud, new growth from lateral buds goes on for a time, not only in the olive, but also in the tomato (Johnston and Dore, 1929) and sugar beet (Brenchley and Watson, 1937).

The development of new shoots in the example shown by Johnston and Dore and in figure 1, speaks against any assumption that the affected plants could not produce growth hormones, although Eaton (1940) was able to increase the growth of cotton seedlings in a boron-deficient medium by the application of indole acetic acid. The problem is: Why are other buds able to produce shoots where the apical bud is not? Aside from its scientific implications, it has immense practical importance. The surviving replacement shoots of tomato and other annual plants arise too late to give fruit, and young trees form a bushy instead of upright type of growth.

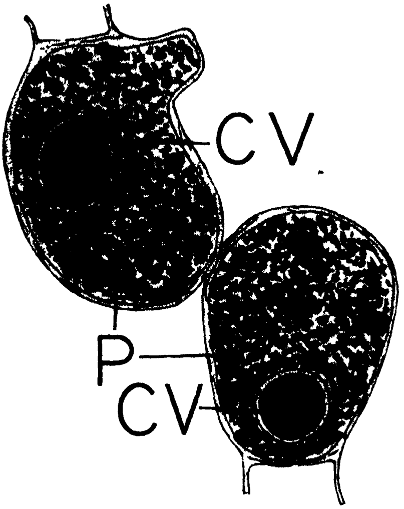


Fig. 2.—Mesophyll cells in an olive leaf showing boron deficiency. *CV*, coacervates of pyridoxin enveloped with lipoid material; *P*, phenolic material which filled the vacuoles and obscured other cell contents. Sudan III and methylene blue stain.

The phenomena exhibited by the development of seedstalks of the sugar beet in relation to boron as reported by Brenchley and Watson have especial interest because the plant is a biennial. If no boron-deficiency symptoms appeared in the first year, the plants produced a healthy single central flowering shoot in the second year without the addition of boron; however, if slight deficiency symptoms appeared in the first year, the shoots produced in the second year were weak and belated unless boron was added when the roots were transplanted. In the latter case the initial injury had not always killed the central bud but the shoot produced from it was weak. If it had been killed, secondary shoots arose around the crown, grew, and flowered freely, without evidence of boron deficiency provided boron was added when the roots were transplanted. Thus the question again arises: Why are the secondary shoots able to grow from roots on which primary shoots showed all the symptoms of boron deficiency and prematurely died?

Growth of the central flowering shoot of the carrot also fails if the boron supply falls below a certain threshold value (Warington, 1940). Necrosis of tissues begins in the region of the growing point of the shoot, though the meristematic apex is not necessarily the first to be involved. The components of the fibrovascular bundles are seriously affected by a lack of boron. On a subsequent page of this paper certain details of the histology and cytology of affected celery plants are presented, and further discussion of the question of the primary meristem will therefore be deferred.

In the present study both cytological and cytochemical investigations were made of boron-deficient olive leaves.

Sections of both fresh and fixed material of healthy leaves, tested with the proper reagents, showed normal structure wherein the cells were free from phenolic and lipoidic substances. On the other hand, an examination of sections of dwarfed, chlorotic olive leaves borne on boron-deficient trees revealed that the cells of palisade parenchyma and mesophyll were replete with flocculent phenolic material which obscured everything except coacervates (fig. 2). When sections were stained with a combination of Sudan III and methylene blue, the enveloping layer of phospholipoid of the coacervate was stained deep pink. Supplementary tests showed that the central mass of the coacervates contained pyridoxine.

When stained with molybdenic acid reagent the surface layer was colored deep blue as a result of its interaction with phospholipoid. This reagent was prepared by the following formula:

Dissolve 6.41 grams of ammonium molybdate in 250 cc of 3*N* sulfuric acid. This constitutes solution A. Dissolve 5.75 grams of sodium bisulfite in 85 cc distilled water. Add 5 grams of 1-amino 2 naphthol 4 sulfonic acid and 5 cc of a 20 per cent sodium sulfite solution. Stir the mixture until the sulfonic acid is dissolved. This constitutes solution B. At the time of using, mix 25 cc of solution A and 5 cc of solution B.

The formation of phenolic aggregates in cells like those here studied is essentially a separation of phases in a physical sense as a result of impaired metabolism in cells affected by pathogens, by viruses, or by the deficiency of an essential element. In healthy cells the dihydroxyphenols (chiefly catechol) are uniformly dispersed in the vacuolar sap, which under the microscope appears optically empty. At pH 5, which approximates that of most vacuolar solutions, half the catechol present is in the oxidized and half in the reduced condition. It is not irreversibly oxidized because there is an equilibrium between the hydrogenases and the dehydrogenases. When the equilibrium is destroyed (by malnutrition, disease, or poisons), the catechol is oxidized to quinones, which may undergo rapid polymerization to more complex compounds. These, being but slightly soluble in the vacuolar sap, appear as flocculent material in various stages of aggregation in the vacuoles. (Reed, 1941). Even though the flocculent material becomes aggregated into globular precipitates (fig. 3, bundle-sheath cells) or into coacervates, the cell may retain its vitality for a time, although the vacuolar solution is impoverished. Both kinds of aggregates may be formed if there is not enough phospholipoid or other desolvant, like lecithin, to form autocomplexes of all the polymerized phenolic material.

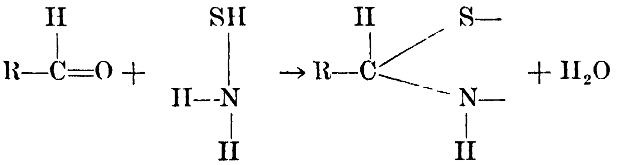
The phenolic compounds, as they lose hydrogen, develop ability for carbon linkages; and the hydrogen enters into the formation of water, and the amount available to redisperse the polymerized phenolic compounds is thereby reduced (Mason, Schwartz, and Peterson, 1945). Everything indicates that the hydrogenase systems in these cells, which could have made hydrogen available, failed to act either because certain phosphorus linkages had been broken or because active —SH groups had been oxidized to disulfides (Reed and Dufrénoy, 1935). The survival of the cell depends upon several factors, such as respiration, illumination, transpiration, and specific characters of the plant.

Certain aspects of respiration may appropriately be discussed here in relation to the physiological equilibriums of plant cells such as those of the olive leaf. In aerobic cells "compensated respiration" depends on the presence of active —SH groups able to release hydrogen as needed to rehydrogenate the dehydrogenated polyphenols or the dehydrogenated dienols. The energy which is liberated in these processes can be utilized by the cell. Compensated respiration therefore depends on the maintenance of the rH potential where cysteine may be in equilibrium with cystine as

$$2\,\mathrm{R{-}SH} \rightleftarrows \mathrm{R{-}S{-}S{-}R} + 2\,\mathrm{H^+}$$

This is generally insured through the presence of glutathione, where the —SH group is stabilized through the polypeptide linkage of the molecule, so that the molecule acts as a reservoir of hydrogen, able to release hydrogen to cystine, which is thereby reduced to cysteine.

Active —SH groups are not likely to exist in the cell in high concentrations, as they are apt to link up with the aldehydic groups of tissue constituents either through a straight —CHO + —SH linkage, or in the case of cysteine through the formation of a thiazoline or of a thiazolidine ring.



Such rings seem to play an important role in nucleoproteins, and denaturation involves the opening of the ring, with liberation of —SH groups, apt to oxidize into —S—S— groups.

When the respiration is "noncompensated" the dihydroxyphenols and other constituents of the cell are subject to further oxidations, resulting in changes of their physical condition (Humphrey and Dufrénoy, 1944).

Returning to the cytological features of the olive leaf, it is important to note that the fibrovascular system showed profound injury where boron was deficient. Eventually the phloem was destroyed. In the earlier stages the phenolic complex in the outer cells showed changes similar to those already described in the palisade and mesophyll tissues. There was first formed a mass of flocculent material in the cells of the bundle sheath. Normally this is an active metabolic system, in which materials are exchanged between the conducting vessels and the centers wherein complex products are manufactured by synthesis of incoming material. The condition shown in figure 3 indicates highly significant early stages in the process of vascular disintegration. The section was stained with acid fuchsin and methyl green. This brought out the fact that various cells were replete with one form or another of oxidized phenolic material. Sections of healthy olive leaves showed none of these alterations; their bundle-sheath cells contained starch. Sieve tubes and companion cells showed premature vacuolization and disruption of their contents. Later the phloem vessels were filled with dense melanotic material which stained deeply with haematoxylin or molybdenic acid reagent (figs. 4 and 5). As conduits for the translocation of solutes, their efficiency was almost completely destroyed. Characteristically in this stage of the process the black necrotic substance did not diffuse into adjacent cells. Its viscosity must be high.

A brief discussion of the origin of the melanotic substance is in order since it is such a constant characteristic of the phloem of boron-deficient plants. Nelson and Dawson (1944) showed how the enzymatic oxidation of catechol to *o*-benzoquinone can be followed (rapidly at pH 7, slowly at pH 4) by hydration of the oxidation product to hydroquinone to hydroxyquinone, which can rapidly polymerize to melanotic compounds. The proposed chain of reactions can be confirmed by measurements of the oxygen uptake and explains why a change in the rate of oxidation occurs at the one-atom stage, when very dilute solutions of catechol are enzymatically oxidized at relatively low pH. The uptake of the second atom of oxygen is conditioned by the hydration of the *o*-benzoquinone.

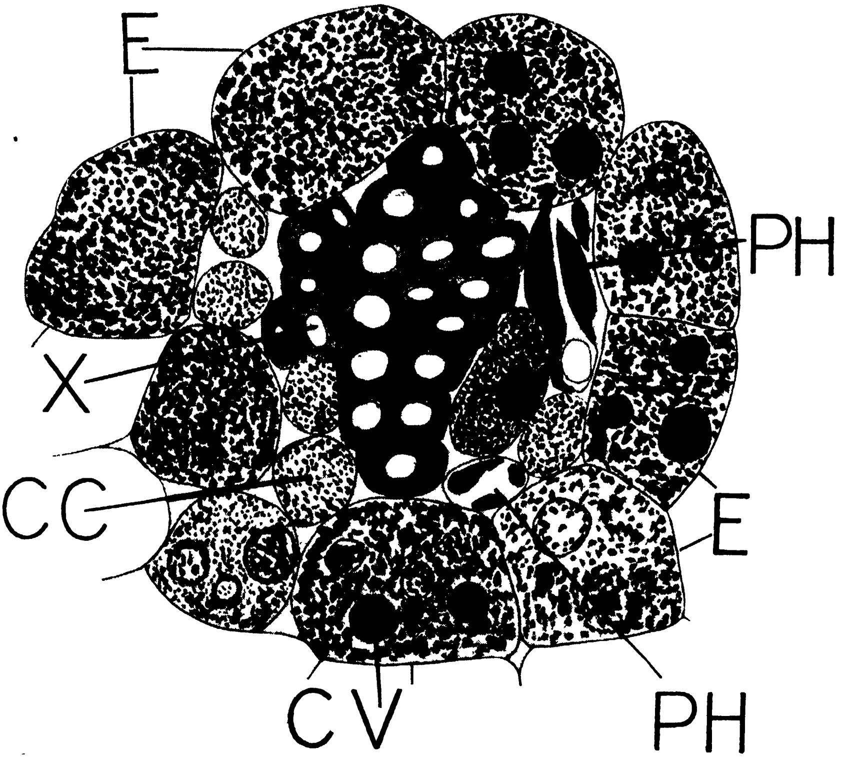


Fig. 3.—A fibrovascular bundle of an olive leaf showing boron deficiency. Derangements of phloem and bundle-sheath cells were evident. *CC*, companion cell; *CV*, coacervate; *E*, bundle-sheath containing phenolic material in various stages of aggregation; *PH*, phloem cells, seriously disorganized; *X*, xylem. Acid fuchsin and methyl green stain.

All evidence concurs in showing that the phenol oxidase which catalyzes the first stages in this process is a copper-containing compound and that it is highly specific for the oxidation of *o*-dihydroxy-phenols such as catechol and

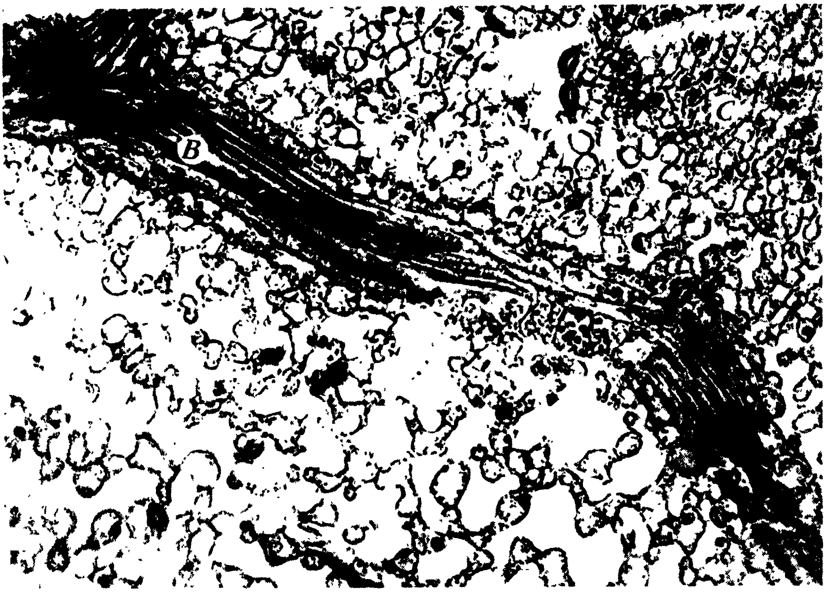


Fig. 4.—Part of a section of an olive leaf which was chlorotic as a result of boron deficiency, showing (*B*) collapse of phloem and (*C*) coacervates in the upper right-hand corner. (× 600.) Molybdenic acid reagent and haematoxylin stain.

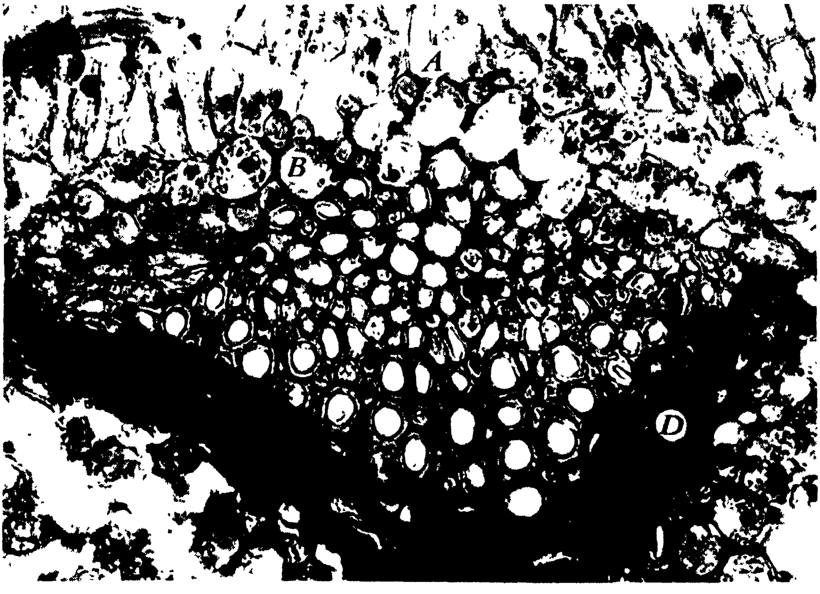


Fig. 5.—Part of a section of an olive leaf which was chlorotic as a result of boron deficiency, showing collapse of phloem: *A*, palisade parenchyma; *B*, endodermal layer; *D*, necrotic phloem tissue. (× 600.) Stained with molybdenic acid reagent and haematoxylin. (Material collected by Professor H. E. Thomas, Jan. 26, 1944.)

pyrogallol. There is no convincing evidence that hydrogen peroxide is formed during the reactions which lead to the formation of the melanotic substance.

Mason, Schwartz, and Peterson (1945) presented spectrophotometric data on the mechanism of the enzymatic oxidation of catechols, whereby it seems probable that *o*-benzoquinone, the initial product of the oxidation, participates in a polymerization to a phenolic chain susceptible to further enzymatic oxidation. Kinetic spectrophotometry of the autoxidation, oxidation in the presence of unsaturated fatty esters, and oxidation in the presence of tyrosinase of 3-*n*-pentadecylcatechol similarly lead to the conclusion that 3-*n*-pentadecyl-*o*-benzoquinone is the initial oxidation product in each case.

To summarize: boron deficiency in the olive results in: (1) the production of growth-inhibiting factors; (2) necrosis of the terminal bud and often of the distal part of the leaf; (3) the production of substances inimical to plastids and consequently limiting carbohydrate production; (4) impoverishment of the vacuolar solution, especially in the leaves; (5) disruption of fibrovascular system through increased oxidative activity, which produces excessive amounts of viscous melanotic substances from quinoids and other compounds flowing through the system; and (6) the production of some lethal material—probably quinoid, but as yet unidentified.

The cytophysiological study reveals that normally the phloem and bundle sheath are metabolic centers as well as conduits for the flow of material. The evidence gained affirms their importance in synthesizing and translocating energy-rich substances, among which energy-rich phosphorus compounds are definable. When phospholipoids are bound to the surface of coacervates, the vacuolar sap is necessarily impoverished in usable phosphorus.

The exact role of boron in maintaining the proper equilibrium in the plant cannot now be definitely stated. There is ample evidence that a small amount is essential for some exceedingly vital cellular processes and that it plays a primary role in the chemistry of the vacuolar solution. In boron-deficient plants the hydrogenase system of the cell fails, owing either to the blocking of the catalyst or to the lack of hydrogen donors, or both.

No valid arguments can be presented for the assumption that boron activity is intimately linked to the functions of cations essential in plant nutrition, though it is known that none of them has completely independent effects in a living system.

Further light on some of these matters will be obtained from data in the next part of this paper.

CELERY

It has been known for some time that cracked veins and hollow petioles are symptoms of boron deficiency in celery (Purvis and Ruprecht, 1937). The present experiments and observations on celery were designed to supplement, and confirm if possible, data on the olive leaf.

In many experiments with celery plants grown in nutrient solutions of highly purified salts, very definite symptoms of boron deficiency were repeatedly obtained. The leaf petioles grew slowly and eventually showed split veins and drying of the central pith, the leaflets were dwarfed, chlorotic, and eventually died at the margins, the roots grew slowly for a time but suffered for lack of organic nutrients from the tops. (Formerly such symptoms could be seen in market celery from certain districts but now, having learned the cause of the difficulty, the growers apply sufficient boron to eliminate the defects formerly seen.) In experiments where no boron was supplied to the nutrient medium, the bud at the apex of the shoot died, whereupon lateral accessory buds put out and made a little growth before the entire plant perished. When 0.05 p.p.m. of boron was supplied, the plants grew well for a time, but sooner or later showed chlorosis, split veins, and failed in other ways to develop into normal plants. Under the experimental conditions, 0.5 p.p.m. of boron was sufficient to produce strictly normal plants.

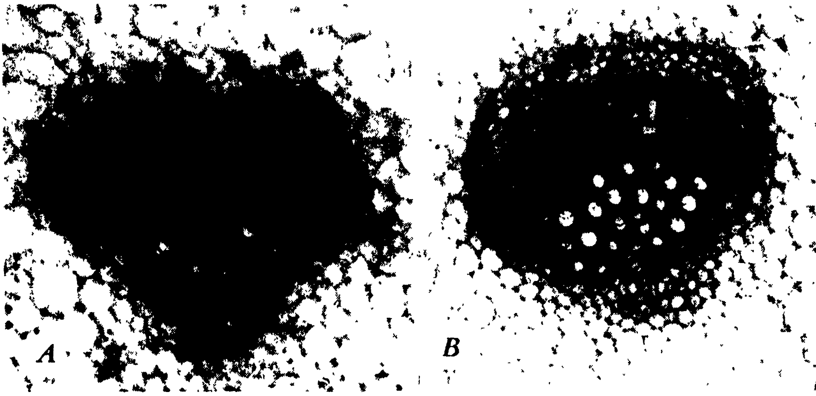


Fig. 6.—Fibrovascular bundles of celery. Free-hand sections after treatment with Gomori reagents: *A*, from healthy plant; *B*, from a boron-deficient plant in which the necrotic phloem contained inorganic phosphates.

Material from plants grown in solutions of highly purified salts was taken to the laboratory for cytological study. Thin sections of the petioles were tested for phosphates by the Gomori and by the molybdenic acid reagent. The cells of the parenchyma and xylem of boron-deficient plants were normal so far as structure and organization were concerned; but the necrotic phloem of boron-deficient petioles had become an unorganized mass of melanotic material in which inorganic phosphate had been trapped (fig. 6, *B*). The intact oil ducts in the metaphloem were similar in all respects to those described by Esau (1936). The dyes which were applied to the fresh sections were more readily absorbed by the injured phloem than by other tissues of the boron-deficient plants, probably on account of greater permeability of the former. Thionine and methylene blue were absorbed and stained the walls of collenchyma and bundle caps, but neither showed marked reduction.

The phloem in boron-deficient petioles was stained purplish pink by phosphomolybdate haematoxylin and brownish purple by phosphotungstic haematoxylin. The former dye also gave the typical blue of the phosphate ion when applied to sections of the boron-deficient petioles.

The parenchyma cells of both lots of celery plants reduced methylene blue, the reduction being somewhat more complete in the boron-deficient petiole sections. The parenchyma cells of healthy and affected petioles reacted somewhat differently to brilliant cresyl blue dissolved in a solution buffered at pH 7. The reduction of the dye was more complete in the healthy petioles. The bundle cap next the fibrovascular bundle was very light blue in contrast to the darker blue of the phloem, which did not reduce the dye.

Additional evidence on the oxidoreducing substances was obtained from tests for phenol oxidase. Boron-deficient sections placed in thydi reagent[4] showed a strong indophenol-blue reaction. Healthy sections, on the contrary, showed none; this difference indicates a stronger oxidase in the affected plants. Sections of affected plants absorbed neutral-red solution much more rapidly and completely than sections of healthy plants.

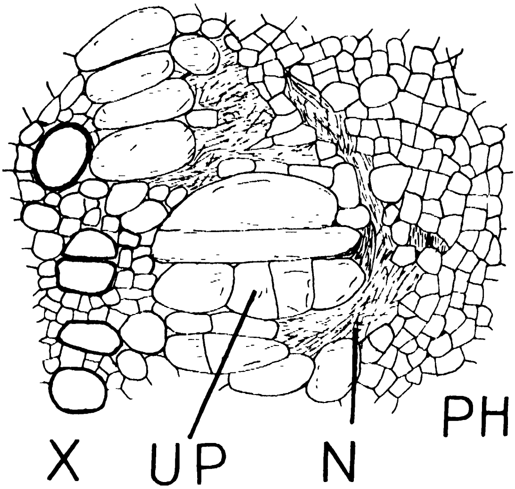


Fig. 7.—Fibrovascular bundle of a celery plant affected by boron deficiency: *PH*, phloem; *N*, early stage of necrosis in the phloem; *UP*, undifferentiated parenchyma; *X*, xylem.

Hypertrophy of parenchyma of affected petioles was commonly observed. The cambial cells were initially elongated in the radial direction and soon divided by transverse walls, but retained the character of parenchyma tissue. Figure 7 represents an early stage before serious derangements had occurred. The development of this undifferentiated parenchyma is responsible for the enlarged vascular strands which are characteristic of boron-deficient celery. It is generally obscured after extensive necrosis of the phloem has produced extruded melanotic material. Eltinge (1936) demonstrated in *Zea* affected by boron deficiency the hypertrophy of parenchyma cells, as well as collapse of mesophyll cells and subsequent necrosis.

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[4] The formula is:

Solution A: Dissolve 0.5 gram thymol in 100 cc water (+ 0.5 cc conc. or strong KOH) on steam bath. This will keep 15 days.

Solution B: Dissolve 0.5 gram *p*-dimethyl-phenylene-diamine-hydrochloride (or *p*-amino-dimethyl-aniline) in 100 cc water. Add a few drops of KOH until rose color disappears, but not enough to turn it blue.

For use: Add a few drops each of A and B to 20 cc water. A blue color should develop. The dye is not permanent and must be made up as used.

Relevant information on this subject was obtained from studies on tomato leaves grown with inadequate supplies of boron. Cells of the palisade parenchyma were two to three times as long as normal, so that the leaflets became thick and curled. Chloroplasts were scarce and had a tendency to assemble near the end, rather than along the lateral walls (fig. 8), and nuclei were sometimes swollen and lobed. The coacervates in the cell vacuoles were, however, the most conspicuous and relevant features of these cells.

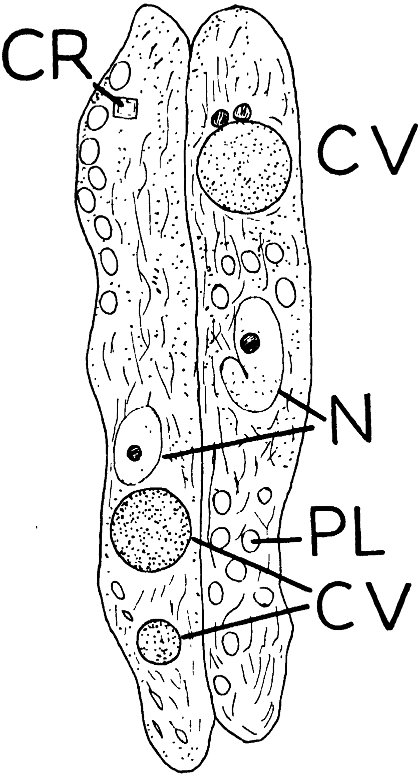

Fig. 8.—Palisade parenchyma cells from the leaflet of a boron-deficient tomato plant. Hypoplasia was shown by the disintegration of cell contents, lobed nuclei, and the presence of coacervates: *CV*, coacervates; *CR*, crystal of calcium carbonate; *N*, nucleus; *PL*, plastids. Safranin and methyl green stain.

If this condition of hypertrophy in parenchyma cells be due to factors which inhibit nuclear division, and cause cells to grow to excessively large dimensions, it can be connected with the disruption of the organic phosphorus complexes either in the leaf or in the stem. In the stem the first visible indications of hypoplasia due to boron deficiency are associated with the enlarged nuclei about which plastids are grouped (fig. 9). The topography of the cell indicates changes in the surface tension and osmotic pressure of the vacuolar solution. In leaves, even those only slightly affected by boron deficiency, the perivascular cells showed much more profound derangements (fig. 10). Mitochondria were unusually abundant; plastids had not developed, probably because of the adsorption of phospholipoids by coacervates had impoverished the vacuolar solution; the nucleus was swollen; and phenolic material was floating in the vacuole. The normal organization was destroyed, not by things absorbed, but by the lack of necessary complexes which in a healthy leaf are derived from the sources of supply.

It has been known since the work of Johnston and Dore (1929) that sugars and starches are more abundant in the leaves and stems of boron-deficient than in those of normal tomato plants. The formation of glucose-6-phosphate is an important step in carbohydrate metabolism representable as follows:

$$PO_4 + \text{glucose} + \text{adenosine triphosphate} \xrightarrow{\text{hexokinase}} \text{glucose-6-phosphate.}$$

If there were a scarcity of adenosine triphosphate, the reaction would be retarded and reducing sugars would predominate. The assumption is supported by the findngs of Johnston and Dore. The inhibition of nucleoprotein synthesis and of carbohydrate transformation gives evidence that some important phosphorus complex was scanty in the celery-petiole and tomato-leaflet cells.

Young shoots which grew from accessory buds after the death of the main shoot of boron-deficient plants afforded material for histological and cytological studies of the apical meristem and embryonic leaves. Longitudinal sections of the apical region (figs. 11 and 12) reveal the extent to which growth and development were disrupted in the meristematic regions. In the apical dome the polyhedral cells containing dense granular cytoplasm and large nuclei had maintained their integrity. Supplies of hexose sugars and amino acids can reach such cells from the subjacent cells. Growth was due to cell multiplication rather than cell enlargement. The equilibrium between oxidation and reduction would be maintained by the presence of adequate hydrogen donors, as shown by the absence of phenolic inclusions. At the base of the primordium the meristem gave rise to cells which expanded rapidly, with simultaneous enlargement of the vacuoles. But indications of hypoplasia were apparent near the base of the primordium, where the contents had contracted and the cells were compressed; necrosis followed. Many of the cells which were able to survive were hypertrophied, a condition resembling that previously observed in the meristematic region of zinc-deficient peach trees (Reed, 1941). It should be noted, however, that the primordia of boron-deficient celery, on account of low oxidase activity, contained a minimum of phenolic compounds.

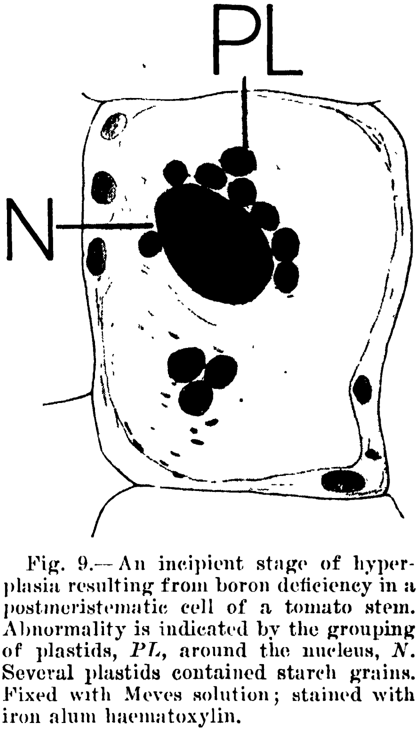

Fig. 9.—An incipient stage of hyperplasia resulting from boron deficiency in a postmeristematic cell of a tomato stem. Abnormality is indicated by the grouping of plastids, *PL*, around the nucleus, *N*. Several plastids contained starch grains. Fixed with Meves solution; stained with iron alum haematoxylin.

Intense symptoms of injury were obvious in the leaf primordia of celery. Cells at the base were greatly enlarged, the separate vacuoles had coalesced into a single large vacuole, and lysis of their contents ensued (fig. 11). Layers of black, necrotic material appeared in the leaf primordia. These replaced the tissue which should have developed into mesophyll and palisade parenchyma, and betokened the oncoming death of the leaf rudiments. Emphasis should be laid on the fact that cessation of cell division and hypertrophy are the first indications of injury in embryonic tissues, whether of leaf or stem (fig. 12).

Figure 13 represents a lateral, not a median, section of another affected stem apex. The collapsed cells (*B*) stained green, the necrotic cells red, with safranin and fast green. (Safranin is a basic dye which is valuable as a protein stain in plants.)

The cells of the primary meristem were generally healthy, and their contents were normal for such cells. Below them hypertrophy was evident, the healthy nuclei absorbed fast green, the injured nuclei safranin, as they do in hypoplastic cells. Surrounding this primordium there was a layer of collapsed cells whose walls stained green, the remains of their protoplasm deep pink. The cells of the leaf primordia were not so seriously deranged except that they contained filamentous mitochondria, had no plastids, and in a few places were collapsed. The derangement of metabolism in epidermal cells was disclosed by the presence of phenolic compounds, either flocculent or granular. The conditions represented only the initial stages of the severe type of injury exhibited in figure 11.

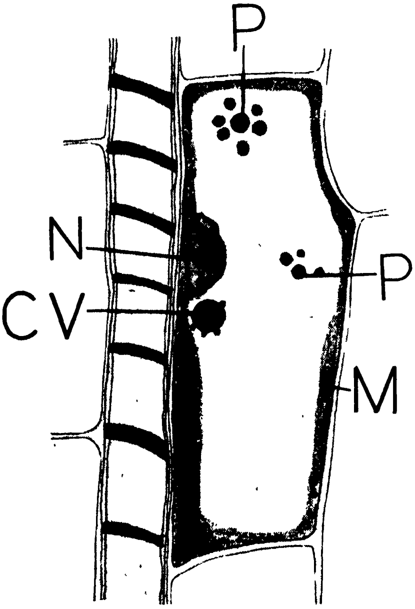

Fig. 10.—A perivascular cell from a leaf of a tomato plant severely affected by boron deficiency: *CV*, coacervate; *M*, mitochondria; *N*, nucleus; *P*, phenolic precipitates. Fixed in zinc chloride plus 2 per cent formalin; stained with molybdenic acid reagent.

A few of the fundamental phases of the metabolism of cells in the primordia of plant shoots may well be reviewed at this point. It is known, for example, that meristematic cells use up the incoming sugar, partly for respiration but mostly for the synthesis of proteins. In either case the active agent in the utilization of incoming material is related to the enzyme system of the vacuole of the cell. The cell protoplasm produces the active agents which pass into the watery solution which fills the vacuole. Much of the physiological activity of the cell is expended in maintaining a proper balance between oxidation and reduction in this reaction chamber. Boron deficiency seems to upset the oxidation-reduction potential in postmeristematic cells, whether in buds or cambial layers, so that dihydroxyphenyl-alanine is no longer protected against irreversible dehydrogenation, but undergoes successive changes, eventually being converted into melanins. These dark compounds are often absorbed by the nuclei and the cell walls, giving a fresh unstained section the appearance of a histological preparation stained with haematoxylin. From our knowledge of the chemistry of melanin formation it seems likely that a transitory red stain is the precursor of the black tyrosine, being first converted into dihydroxyphenyl-alanine, then eventually to melanin. When in excess in the roots of plants like beets, turnips, and radishes, it gives rise to the conditions commonly known as black heart.

This problem of the relation of boron to the growth and development of the apical meristem has been studied in detail by Beckenbach (1944). Tomato plants which had received 0.5 p.p.m. of boron until the first blooms appeared, thereafter received differential boron treatments—0, 0.005, 0.5, and 10.0

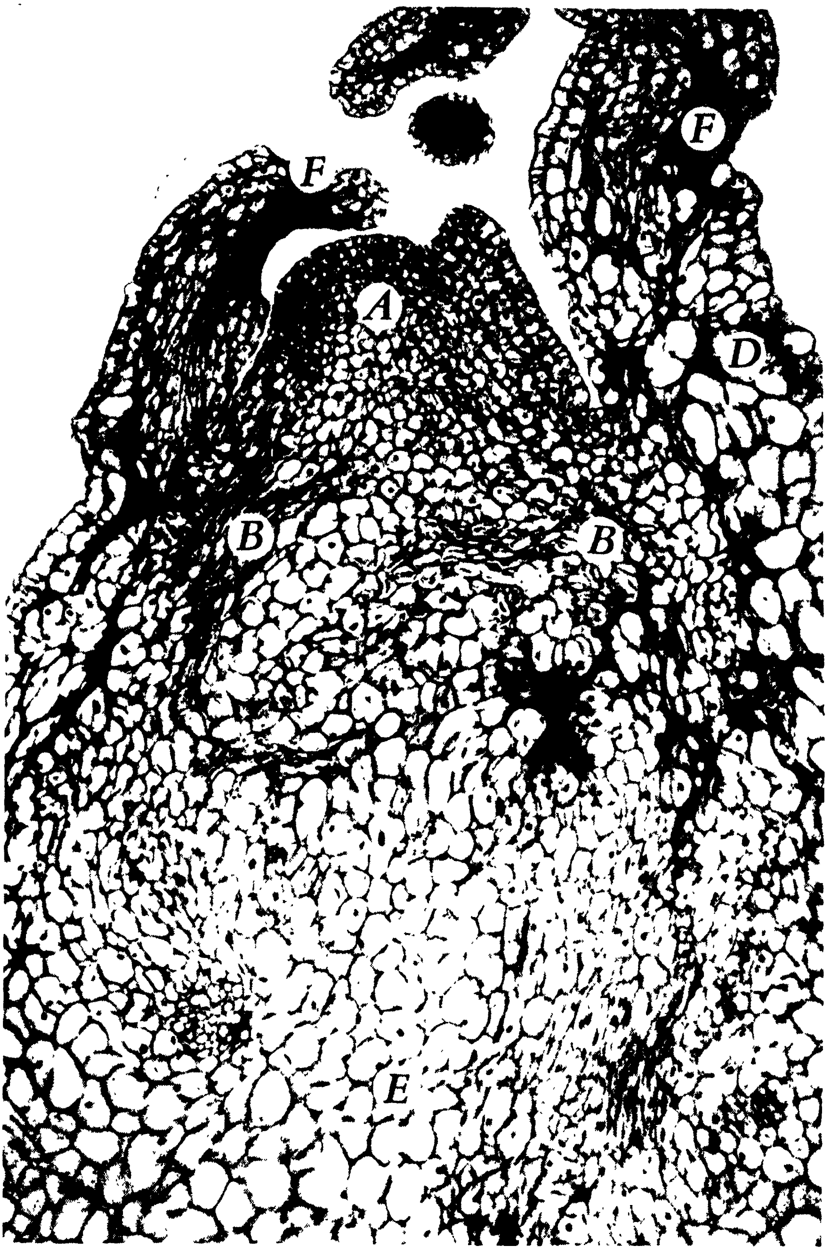

Fig. 11.—Apical region of a shoot of a boron-deficient celery plant. Parenchyma cells beneath the apical meristem were hypertrophied and (at the left) had collapsed. *A*, Nuclei hyperchromatic, cytoplasm vacuolated, vacuoles generally polar; *B*, collapsed cells whose contents were stained deep pink; *D*, cells which contained filamentous mitochondria but no plastids; *E*, hypertrophied cells; *F*, necrotic tissue. Stained with safranin and methyl green.

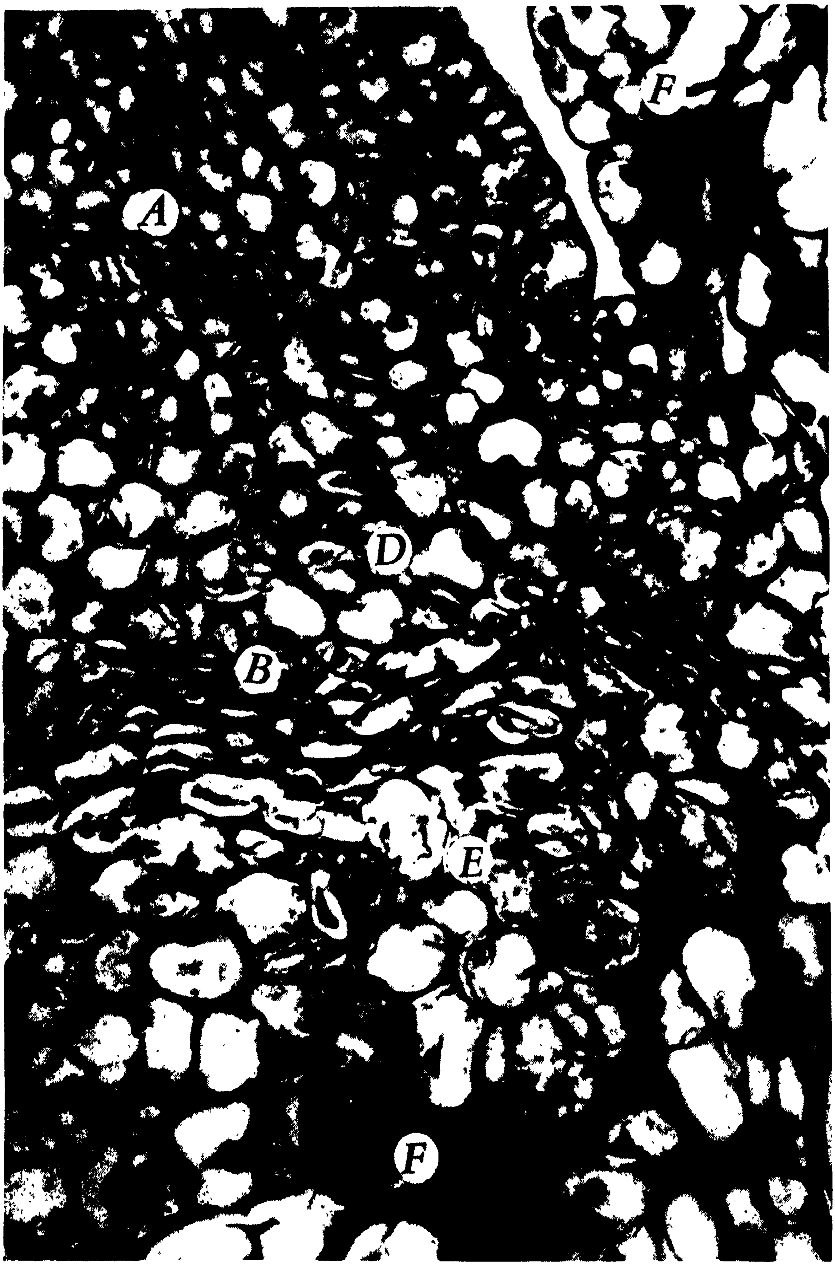

Fig. 12.—Cytological features of the postmeristematic region of a celery shoot showing effects of boron deficiency: *A*, vacuolization and incipient collapse of cells; *B*, collapsed cells; *D*, cells containing large central vacuoles, hyperchromatic nuclei and filamentous mitochondria; *E*, hypertrophied cells; *F*, necrotic tissue. (×445.) Haematoxylin stain.

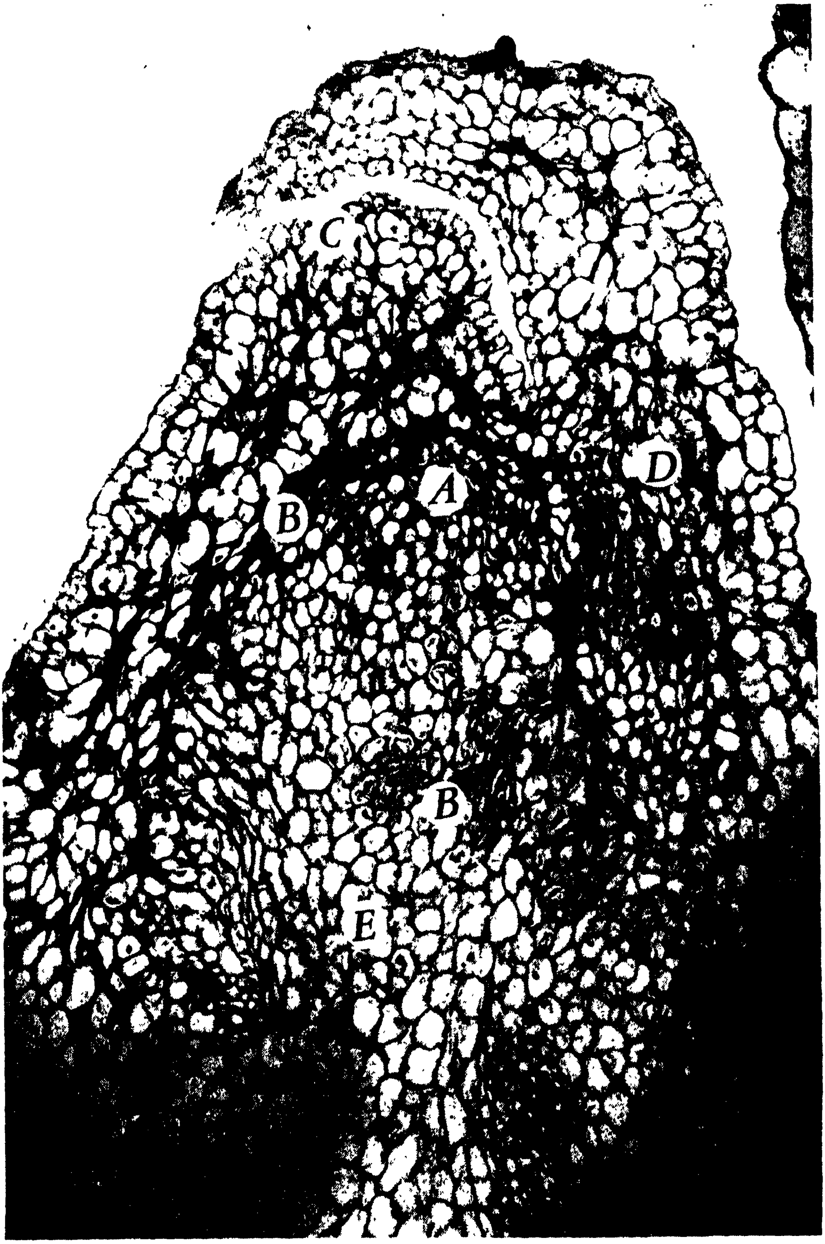

Fig. 13.—Apical region of a shoot of a boron-deficient celery plant; lateral section shows conspicuous layers of collapsed, necrotic cells: *A*, cells apparently healthy; *B*, collapsed cells whose contents were stained deep pink; *C*, cells which contained flocculent or granular phenolic compounds; *D*, cells which contained filamentous mitochondria, but no plastids; *E*, hypertrophied cells. Stained with safranin and fast green.

p.p.m. From an earlier stage the milliequivalent proportions of nitrate, phosphate, and sulfate in the cultures were also varied. A week after boron was withheld, growth was greatly retarded in all series, except where phosphate was high and both nitrate and sulfate were low. It is significant that plants which received no boron and no phosphate became brittle and bushy at the growing point, while injury from boron deficiency was less severe when phosphate was high. When growth was rapid owing to the presence of a high concentration of nitrates, the requirement for boron was high. Plants which received high phosphate were taller but had fewer side shoots; this indicates the dominance of the apical meristem over lateral buds. The rate of growth of the apical meristem was rather uniform in most cultures where the plants received boron, regardless of the amount added, if the nitrate supply was adequate. The linkage with nitrates suggested that boron is essential for normal protein synthesis through its effect on the utilization of carbohydrates. The number of meristems 1 inch or longer per plant did not vary in proportion to the amount of boron supplied.

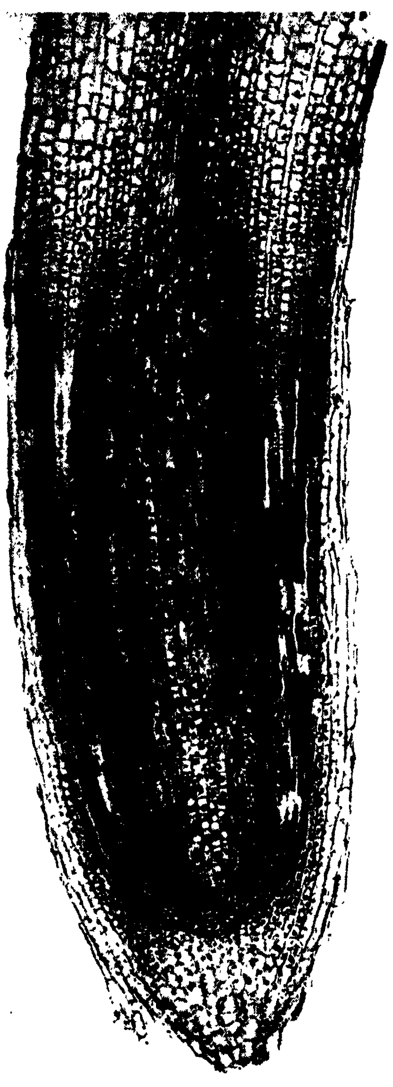

Fig. 14.—Apical region of a healthy celery root. (× 130.) Acid fuchsin and methyl green stain.

Reed and Dufrénoy (1935) showed that the ability of the meristem of the orange shoot to maintain its rate of growth can be correlated with the mobilization of zinc in the meristem at the expense of postmeristematic cells. When supplies of an essential element fall below a minimum, distortion of the growth and development occurs, but the cells compensate for the deficiency in many ways—for example, by an abeyance of the expensive synthesis of new nucleoprotein, hydration of biocolloids, and accelerated respiration, all of which contribute to delay growth and differentiation.

Root tips from celery plants grown in nutrient solution were crushed on a slide, treated with the molybdenic acid reagent, and examined under high magnification. Absence of blue color in the roots of healthy plants indicated a low level of phosphate compounds, since they had flowed to sinks in the shoots and leaves. The dark blue of the stele of boron-deficient roots, on the other hand, indicated a high level of phosphate compounds; the extra-stelar

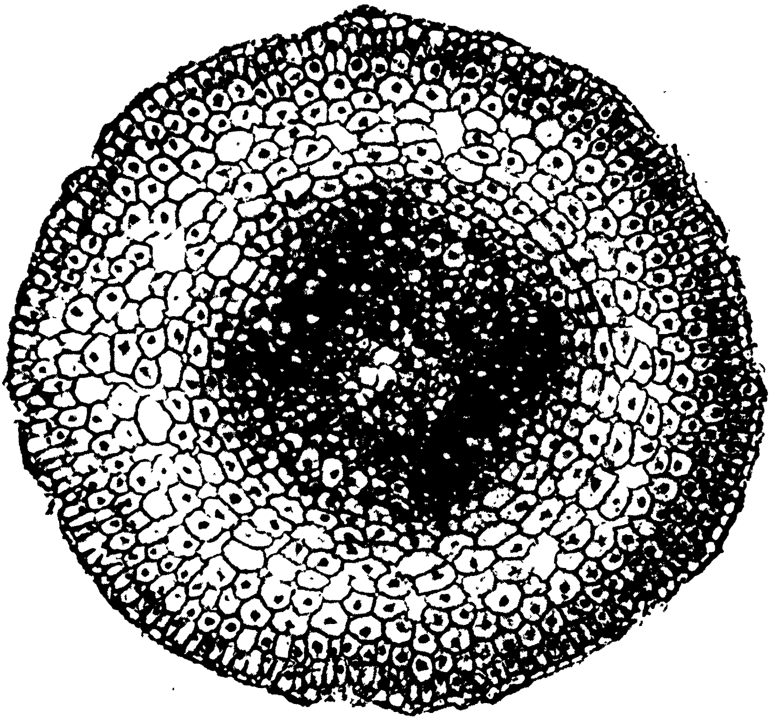

Fig. 15.—Transection of the postmeristematic region of a healthy celery root. (x 185.) Acid fuchsin and methyl green stain.

tissues were faint blue. The endodermis and pericycle formed a definite boundary between the two zones. The boron-deficient roots released phosphates into the reagent after a few hours but none was observed to come from the healthy roots. Within this cylinder, the stele of boron-deficient roots had a high level of energy-rich phosphorus compounds, giving its cells the ability to maintain a low residual oxidase activity because the phosphate complexes could act as dehydrogenases. Outside of this cylinder, the cortical cells were

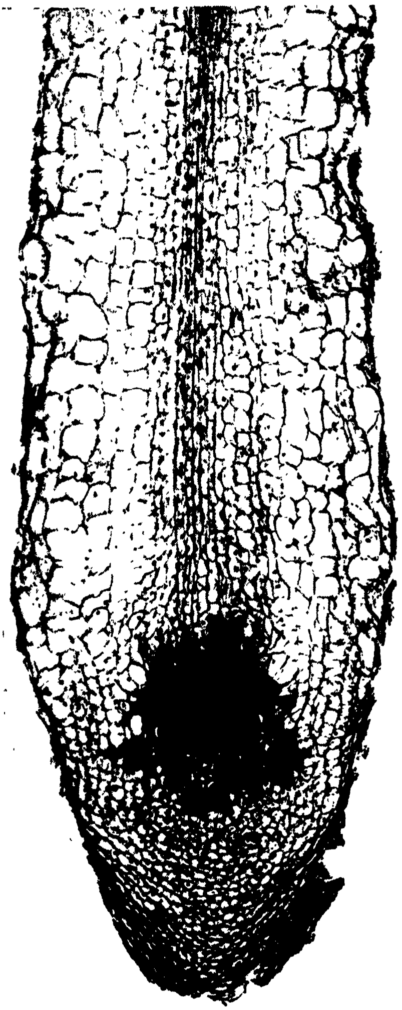

Fig. 16.—Apical region of an affected celery root from a boron-deficient plant. The meristematic region was the site of hypertrophied and necrotic cells. (× 130.) Safranin and fast green stain.

relatively poor in such compounds but rich in catechol and catechol oxidase. Polyphenol oxidase was present in the cortical cells of the boron-deficient roots. It was released abundantly by these roots; and when the cortex was sectioned, blue-purple deposits were found in the intercellular spaces. No definite colored material was seen in the cortex of healthy roots nor was there much evidence of oxidase which had been released. No evidence of phenol oxidase in the stele was observed in either case. These results therefore demonstrate that oxidase is more active in the affected roots and that the phosphates in them do not enter into complexes, but rather remain as phosphate ions. They also show that the cells in these tissues of boron-deficient plants became, to a varying extent, more permeable to the phosphate ions, which normally pass slowly through cytoplasm.

Verification of the above observations was obtained from a study of healthy and affected roots which had been fixed in Nemec's solution and stained with fuchsin and methyl green, with safranin and fast green, or with haematoxylin after being sectioned. Cells of the apical meristem of healthy roots are cubical or polyhedral and contain nuclei and dense protoplasm, which were stained by methyl green (fig. 14). Only the nucleoli were stained with fuchsin. The stele in the postmeristematic region also absorbed methyl green; this indicates that the cell contents were well supplied with complex synthates and that the water phase was well distributed through the protoplasmic material (fig. 15). The thinness of the walls is evidence that they were under tension as a result of the growth of cell contents. The cortical cells were likewise healthy; their walls were thin and somewhat rounded at the corners. Only the outer layers were stained with safranin.

The cells of boron-deficient roots show several contrasts to the foregoing conditions. Figures 16 and 17 indicate some of the derangements which were evident when sections of the roots had been stained by the technique used for the healthy roots. The undifferentiated cells of the apical primordium showed few abnormalities, but the stele in the postmeristematic region showed evident injury. A thickening of cell walls indicated excessive hydration of cellulose and related substances. The young sieve tubes were full of material stainable with fast green, and older collapsed cells were densely stained with safranin. The oil ducts in the phloem were intact though the rest of that tissue was necrotic. Haematoxylin also stained the stele intensely. Outside the endodermis and pericycle, the cells of the cortex appeared healthy at the stage at which this material was collected, though ultimately it became gelatinous and the roots perished. The cell walls were thin, vacuoles clear, nuclei stainable with safranin or haematoxylin, and prochromosomes clearly evident.

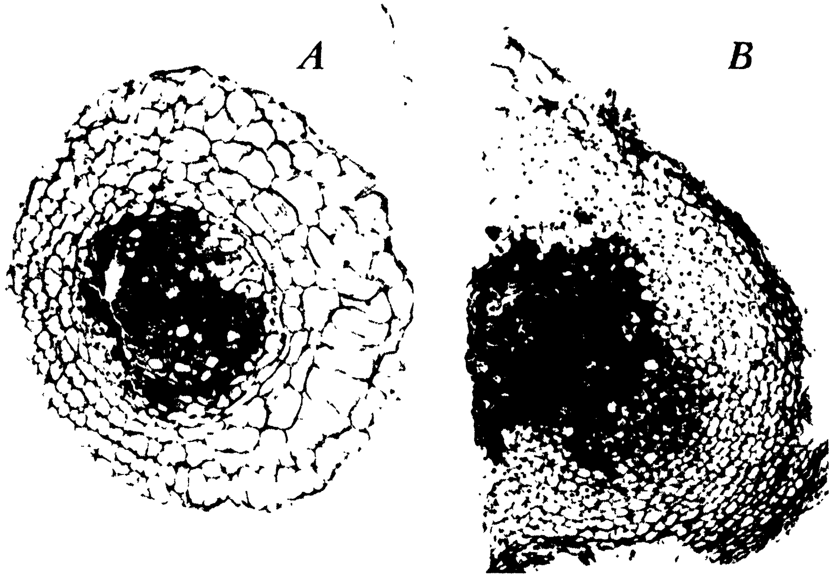

Fig. 17.—Transections of the postmeristematic region of affected roots from boron-deficient celery plants: *A*, stained with safranin and fast green; *B*, stained with haematoxylin. (× 185.)

RADISH

The specific symptoms of boron deficiency in the radish are retarded growth, chlorotic leaves, distorted roots, and black necrotic tissue in the interior of the roots. H. E. Thomas brought from certain market gardens, specimens showing those symptoms. These served as a source of material for laboratory studies. Subsequently plants were raised in iron tanks containing nutrient solutions whose boron contents were 0, 0.05, 0.1, and 0.5 p.p.m. When harvested, the plants from solutions containing boron had produced flowers, but root development in the solution cultures was not so good as it would have been in soil. The data in table 1 show, however, that boron is necessary for the complete development of the plant, though the amounts added were small. Without boron there was a preponderance of yellow leaves, few fleshy roots, and no flowers.

Scripture and McIlargue (1945) concluded from their study of the radish plant that boron is evidently essential for its normal carbohydrate metabolism.

The opportunity for biochemical tests on the plants was so limited that no definite conclusions could be drawn. However, the rootlets of radish plants from zero boron solutions had a stronger oxidase activity, when tested with the thydi reagent, than those from healthy plants from the 0.5 p.p.m. boron solutions, a result confirming that obtained with celery roots. The case was somewhat different, however, when sections of the fleshy roots were examined with the same reagent. In the roots it was quite evident that there was more polyphenol oxidase and more pyridoxine in plants which received 0.5 p.p.m. of boron than in those which had received no boron.

TABLE 1

RELATION OF BORON TO GROWTH OF RADISHES IN NUTRIENT SOLUTIONS

(Germinated June 27, 1944; installed in tanks July 19, 1944; harvested August 12, 1944)

Tank	Boron	Total plants	Plants whose leaves were		Plants having		Fresh weight		Tops / Roots
			Green	Yellow	Fleshy roots	Flowers	Tops	Roots	
	p.p.m.	*number*	*number*	*number*	*number*	*number*	*grams*	*grams*	*ratio*
1A	0.00	24	17	7	6	0	252	160	1.58
1B	0.00	24	13	11	5	0	240	125	1.92
2A	0.05	24	22	2	20	3	620	318	1.95
2B	0.05	24	18	6	14	7	790	212	3.73
3A	0.10	24	21	3	8	6	730	200	3.65
3B	0.10	24	19	5	8	8	728	168	4.33
4A	0.50	24	22	2	16	8	660	206	3.20
4B	0.50	24	22	2	11	9	760	260	2.15

Petioles of the radish leaves were examined for the presence of inorganic phosphate by the Gomori method. The walls of the xylem and phloem tissues of healthy plants were blackened but their contents were not. In boron-deficient plants, the nuclei of the cortical cells were light gray and clear; the brownish-black color of the disorganized contents of the phloem cells indicated that the cells contained inorganic phosphate ions. The evidence thus far obtained leads to the conclusion that the deficiency of a microelement like boron is coupled with an excess of phosphatase which can hydrolyze phosphorous esters.

SUNFLOWER

Several new aspects of the boron-deficiency problem have been gained from a study of the sunflower, which is known to have a definite and rather high requirement for boron. Its rapid growth and adaptability to experimental technique have made it a useful plant for many kinds of physiological investigations.

In the present study the plants grew from the seedling stage in nutrient solutions of purified salts which differed only in the amounts of boron they contained. Plants in solutions which received no boron ceased to grow after two or three leaves had been formed, and eventually perished. Plants in solutions which received 0.5 p.p.m. of boron at the outset and additional doses as the original supply was exhausted were thrifty, had good foliage, produced flowers, and matured seeds. Plants in the intermediate concentrations made limited growth and eventually showed chlorosis and thickened veins in the leaves.

The data in table 2 give further indication of the essentiality of boron. The experiment was conducted in large cast-iron tanks of aerated nutrient solutions, under favorable illumination in the greenhouse. The concentrations of boron supplied are given in the first column of table 2. The green weights of tops and roots in concentrations of 0.05 and 0.10 p.p.m. of boron were not significantly different, an indication that both concentrations were below some threshold value needed for the synthesis of a substrate for growth. Colwell (1943), who estimated the response of sunflowers to boron by an index of age values, found a similar equivalence of growth for 0.05 and 0.10 p.p.m. boron. Johnston and Dore (1929) likewise found no differences in the height growth of tomato plants for the first 3 weeks in culture solutions receiving 0.011 and 0.55 p.p.m. boron. Subsequently, growth in the lower concentration ceased, but in the higher it continued although at a slightly decreased rate.

TABLE 2

RELATION OF BORON TO GROWTH OF SUNFLOWERS IN NUTRIENT SOLUTIONS

(Planted October 12, 1944; harvested November 11, 1944)

Boron	Total plants	Average fresh weight		Tops / Roots
		Tops	Roots	
p.p.m.	*number*	*grams*	*grams*	*ratio*
0.00	19	4.3	3.1	1.4
0.05	24	29.2	6.7	4.3
0.10	21	29.0	7.1	4.1
0.50	19	40.5	10.0	4.1

The data in table 2 also show a similarity in the ratio of weight of tops to roots in all except the plants from solutions which received no boron. Since root growth bears an intimate relation to the supplies of synthates furnished by the leaves, boron must have some important rôle in their function, a conclusion supported in several ways by an inspection of figure 17.

Histological Symptoms of Sunflower Stems. The subapical region of affected sunflower plants, like those in figure 18, *B*, was hypertrophied by the formation of a thick layer of large-celled undifferentiated parenchyma between xylem and phloem (fig. 19), similar in several respects to that in boron-deficient *Vicia faba* (Warington, 1926), *Brassica* (Löhnis, 1940), and tomato (Johnston and Dore, 1929). The swelling of the sunflower stems became conspicuous after the death of the apical bud. It had some histological resemblance to the proliferated tissue of certain plant galls resulting from insect punctures—tissue which Küster (1930) designated as secondary. Walker (1943) has clearly described this phenomenon in *Beta* and *Brassica* affected by boron deficiency.

The cells of the undifferentiated parenchyma in sunflowers were initially hexagonal in transverse section but soon became elongated in the radial direc-

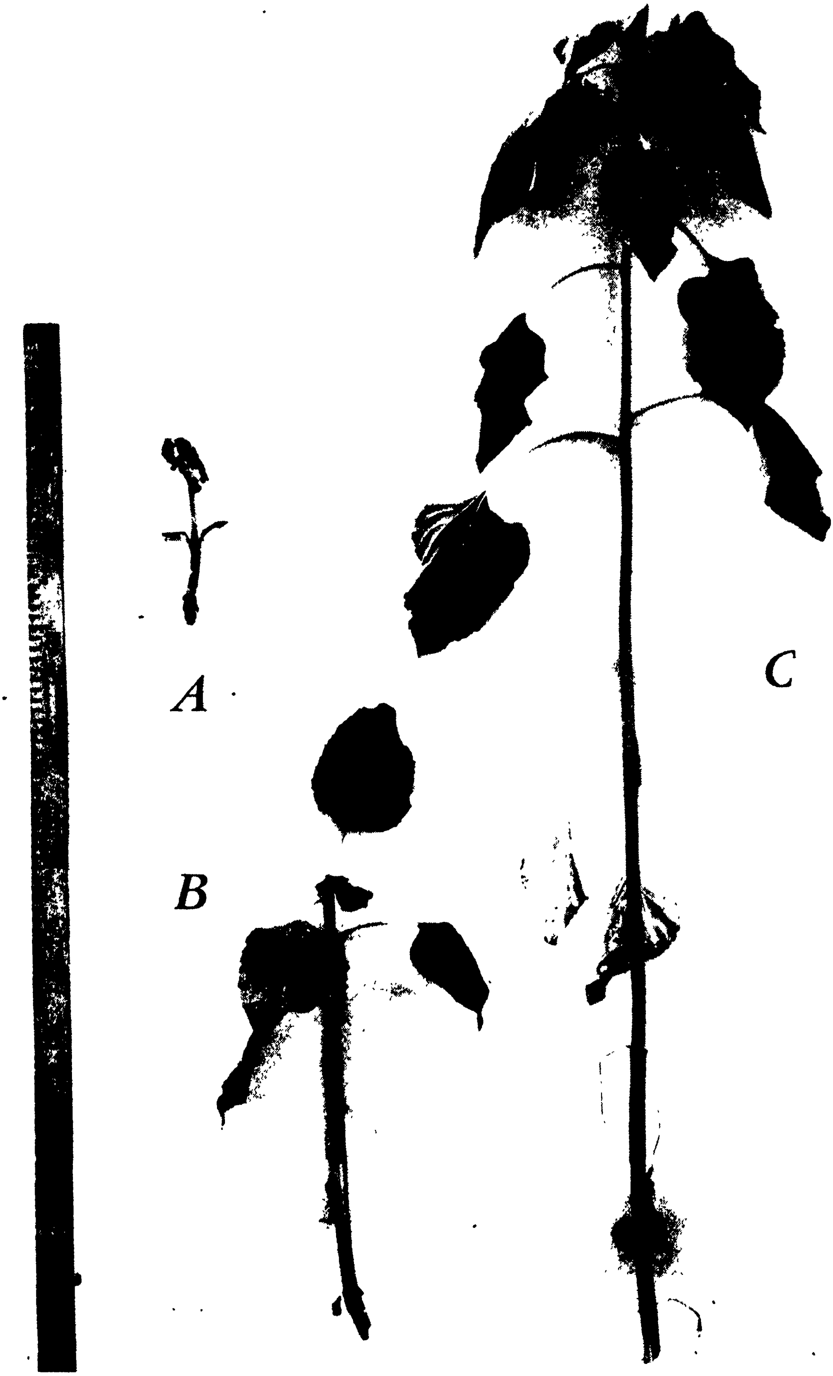

Fig. 18.—Sunflower plants from solution cultures in the greenhouse: *A*, from solution without boron; *B*, from solution containing 0.05 p.p.m. boron; *C*, from solution containing 0.5 p.p.m. boron. (The leaves wilted while the photograph was being made.)

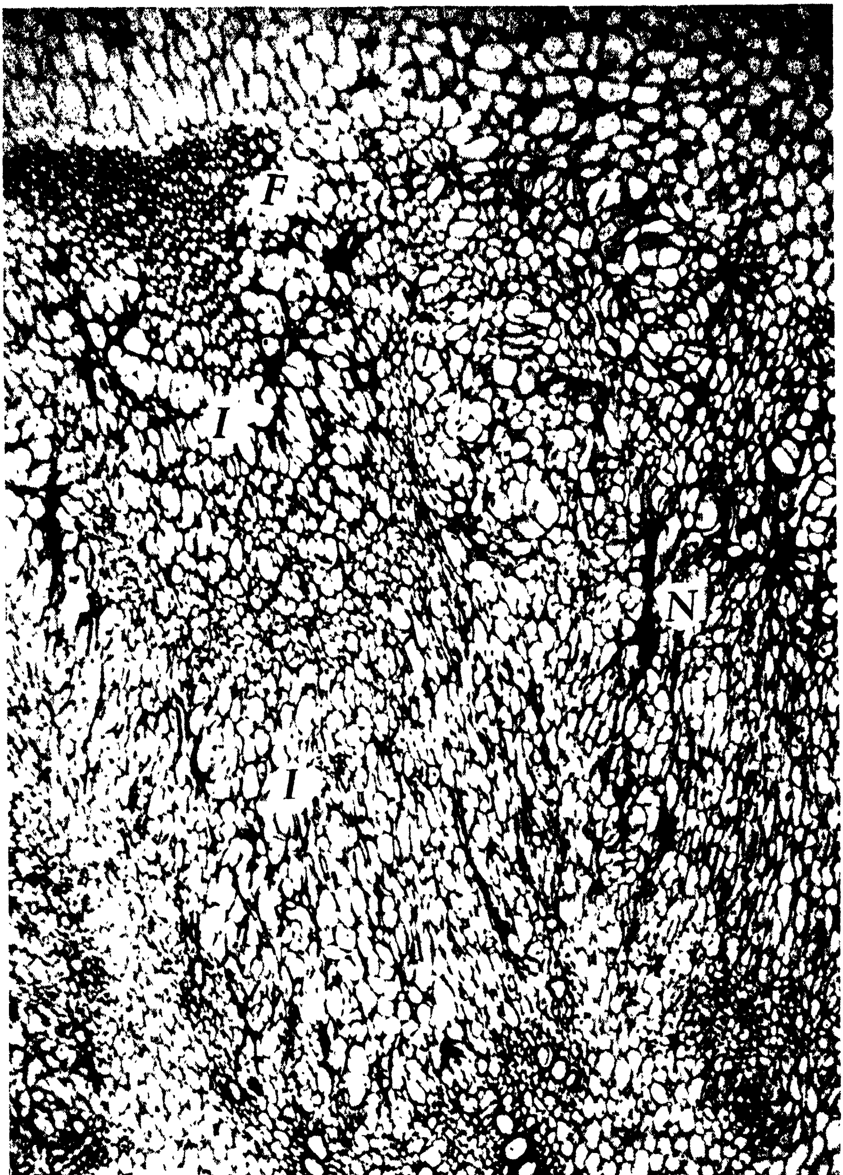

Fig. 19.—Section of a sunflower stem showing later stages of boron deficiency in which there is a thick layer of undifferentiated parenchyma: *F*, phloem; *I*, undifferentiated parenchyma; *N*, necrotic tissue; *W*, xylem. (× 70.) Safranin and fast green stain.

tion. Their nuclei were flattened ovoids lying adjacent to the parietal cytoplasm, most of the vacuoles were optically empty, though a few contained flocculent material which was stainable with phosphomolybdate haematoxylin. Neutral red was not readily absorbed by living cells, but methylene blue was quickly absorbed, an indication that the vacuolar solution was acidic and low in hydrogenases. In more advanced stages of hypertrophy there were radial lines of crushed cells in the cross sections, probably due to tensions set up by high osmotic pressure of the rapidly growing tissue. Observations of Johnston and Dore (1929), Haas (1932), and others support the idea that the enhanced turgidity of similarly affected tissues may be due to a higher content of sugar and other solutes unutilized in the growth process. When, eventually, the cytoplasmic integrity of the larger cells was destroyed by proteolysis, or simply by a disequilibrium between volume and surface, there would not be enough wall pressure to maintain their form, and collapse would ensue.

The cytological studies on the effect of boron deficiency in olive and celery had some reference to the phosphorus compounds, but boron-deficient sunflowers proved to be more suitable plants for a study of the relation of phosphate metabolism to the boron supply.

As an introduction to the problem, the percentages of total and of inorganic phosphorus in sunflower stems and leaves in two parallel experiments were determined. The determinations give a rough, over-all picture of the relations between the two forms of phosphorus in plants which received various amounts of boron (table 3). Each set of plants had grown 70 days in 15-liter tanks of nutrient solutions containing 0.00, 0.05, 0.10, and 0.50 p.p.m. of boron, under conditions similar to those previously described. When the healthy plants in the higher concentrations of boron were in flower, the stems and leaves of each set were harvested, separated, and dried. The dried samples were ground and analyzed by J. Katz. Total phosphorus was determined by the dry ignition method with $Mg(NO_3)_2$. The inorganic phosphate was extracted from aliquots of the dried samples with 2 per cent acetic acid. Phosphate was determined colorimetrically by Allen's method.

The data (table 3) consistently show a greater percentage of phosphorus compounds in the dwarfed chlorotic plants which had inadequate boron. The larger, healthy plants which received adequate amounts of boron diluted the inorganic constituents according to a well-known principle.

Of greater importance is the ratio between inorganic and total phosphorus in the several lots of plants. The analyses of stems show that over 90 per cent of the phosphorus in the zero-boron plants was inorganic phosphorus and that with increasing amounts of added boron, the ratios of inorganic to total phosphorus decreased to about 50 per cent. This is strong evidence of higher phosphatase activity in the tissues of the affected plants, which could result in the release of the energy of phosphate esters for the maintenance of the plants. Albaum and Umbreit (1943) demonstrated an increase in inorganic phosphorus beginning about the seventy-second hour of growth of the oat embryo concomitant with gradually increasing metabolic activity.

The functional relation between phosphate and boron described by Beckenbach (1944) was discussed earlier in this paper, but may be mentioned here in considering the results of these analyses.

The phosphate content of healthy is less than that of affected stems, as shown by relatively simple tests with molybdenic reagent on sections of fresh material. The characteristic blue color appeared only in the hypodermal collenchyma when sections of healthy stems (0.5 p.p.m. boron) were immersed in this reagent, but the blue color appeared in the cortex and undifferentiated parenchyma when sections of stems of boron-deficient plants were similarly treated. Moreover, the reagent in which the sections were immersed was also blue, indicating that they contained diffusible phosphates.

Confirmatory results were obtained by application of the Gomori test on fresh sections of the postmeristematic regions of stems. Healthy material contained little of the black substance except in the walls of xylem and phloem elements, which probably represented phosphate in transit. Sections of boron-deficient plants tested in the same way also exhibited an intense brown-black color in cortex, phloem, and xylem and, in addition, similar (though weaker) color in the undifferentiated and in the central parenchyma. Not only were the cell walls black, but the cells contained minute black granules. Intact phloem elements (chiefly sieve tubes) and cortical parenchyma contained large numbers of the same type of black granules. Comparative tests on similar experimental material which grew in the autumn when illumination was weak, verified the foregoing observations.

TABLE 3

TOTAL AND INORGANIC PHOSPHORUS IN 70-DAY-OLD SUNFLOWER STEMS AND LEAVES AS PERCENTAGES OF DRY WEIGHT

Boron supplied	In stems			In leaves		
	Total phosphorus	Inorganic phosphorus	Inorganic P / Total P	Total phosphorus	Inorganic phosphorus	Inorganic P / Total P
p.p.m.	*per cent*	*per cent*	*ratio*	*per cent*	*per cent*	*ratio*
0.00	0.90	0.83	0.92	0.69	0.55	0.80
.05	.40	.31	.77	.48	.32	.67
.10	.49	.41	.84	.49	.35	.71
0.50	0.29	0.15	0.52	0.37	0.23	0.62

Summing up these histochemical tests we may say that the boron-deficient sunflower stems contained more ionic phosphate than the healthy and that the excess was chiefly in the parenchyma of the cortex and undifferentiated layers. Since they are active metabolic tissues it is significant that they were the site of such rapid phosphate turnover in the affected plants.

A paper by Yin (1945), which came to hand after the foregoing observations were made, corroborates these conclusions by demonstrating a high level of phosphatase activity in the meristematic tissues, the outer layers of the cortical parenchyma, and the phloem elements. He rightly emphasizes the importance of phloem elements in view of their role as conduits for the transport of organic materials and of phosphorus compounds.

The enhancement of phosphatase activity in stems of boron-deficient sunflowers was demonstrated by the use of Willmer's modification of Gomori's test (Willmer, 1942). Fresh sections of the apical regions of healthy and boron-deficient plants were used. The latter showed distortion and incipient necrosis. The sections, after fixation and hydration, were transferred to a solution composed of 2 per cent $CaCl_2$, 1 part; 2 per cent barbital, 2 parts; 2 per cent sodium glycerophosphate, 2 parts; water, 5 parts; in this they were incubated at 40° C. There was no indication of phosphatase in the healthy stems, but the blackened proxylem and protophloem of the affected stems gave definite indication of phosphatase. Similar tests conducted on sections of petioles also demonstrated that the phloem and xylem of affected plants were richer in phosphatase than the healthy.

It is important to ascertain whether such processes as glycolysis, oxidoreduction, and the transformation of phosphorus compounds can be linked up. The postmeristematic region of the sunflower stem obviously affords suitable material for such an inquiry. If the oxidase activity in such tissues is below normal and sugars are being consumed at a low rate, we may reasonably infer that the activity of the phosphatase is noncompensated by the catalysts which, coupled with oxidation, could otherwise build up phosphate esters. Vogler and Umbreit (1942) found that the oxidative phase was accompanied by phosphate fixation, and the reductive phase of carbon fixation by a release of phosphate.

In pursuance of this idea, I investigated the ability of fresh sections of sunflower stems to reduce brilliant cresyl blue, Bindschedler's green, and methylene blue in sealed hanging drops. Brilliant cresyl blue dissolved in barbiturate buffer at pH 7.1 was reduced with the formation of a precipitate only by sieve tubes and undifferentiated parenchyma of boron-deficient plants. In healthy material, on the other hand, reduction was observed in sieve tubes, bast, and xylem parenchyma, with the formation of precipitates. This dye penetrated the vacuoles of collenchyma cells of healthy, but not of affected plants. It penetrated and stained the nuclei of healthy pith parenchyma, but was not reduced. Bindschedler's green was slightly reduced by the fibrovascular bundles. Methylene blue was reduced by the undifferentiated parenchyma of both healthy and affected plants but not by the phloem of either healthy or affected plants. The hydrogenase activity in the affected plants is therefore confined mainly to the phloem and undifferentiated parenchyma, where, as shown above, there was the greatest release of phosphate, accompanied by a release of energy which would be available for maintaining the work of the cell.

The oxidases of the stem show a distribution which is significant for the cytochemical appraisal of healthy and affected plants. Fresh sections of stems were immersed in thydi reagent in watch glasses and transferred, after the color appeared, to slides, and examined under the microscope. The indophenol blue formed more rapidly in sections of affected plants which, although they had received 0.05 p.p.m. boron, showed typical deficiency effects, indicating a higher level of oxidase activity than the healthy plants. In the healthy sections the blue retained was principally in phloem elements; the central pith cells were initially blue, but lost the color by diffusion. The phloem elements, the adjacent undifferentiated parenchyma, and the pith of the boron-deficient plants were deep blue. Upon longer exposure the cortex absorbed the thydi reagent, but the blue color was very faint.

Similar sections were tested for oxidizing substances by a solution containing 100 p.p.m. of α-naphthylamine. There was no color change in sections of healthy plants, except in the bast fibers; whereas in the boron-deficient there was an intense reddish-purple color only in the vacuoles of undifferentiated parenchyma cells between phloem and xylem. Supplementary tests were made with benzidine base slightly acidified with acetic acid, which, upon oxidation, gives a brown-black color. The fibrovascular bundles of healthy and affected stems were stained dark brown after 24 hours, and the undifferentiated parenchyma of the latter was dark lavender.

These observations indicate that the stems of boron-deficient plants had a higher level of oxidase activity than normal. The principal site was in the fibrovascular and undifferentiated parenchyma tissues, with a distinct, though lower, level in the cortex.

The equilibrium between phosphatase and polyphenol oxidase would be continually shifting as the injury to the plants became more severe, parallel with correlative changes in other activities which would tend to minimize the effects of derangements in nutrition. The ways in which the energy released from phosphate esters is utilized cannot at present be stated.

Further information on the condition of the cells of healthy and of affected stems of sunflower plants was obtained from observations on fresh sections of the postmeristematic region which were treated with certain acidic or basic dyes. The technique has been profitably employed by many investigators, since modern research has elucidated the nature and chemistry of the dyes.

The acid dyes employed were vital red, Congo red, Martius yellow, acid fuchsin, and eosin; the basic dyes were methyl green, methylene blue, neutral red (weakly basic), safranin, and Nile blue A.

Congo red and eosin gave the most definite reactions; acid fuchsin faded unless a few drops of citric acid solution were added, giving it a reaction of pH 3.6. The other dyes were dissolved in Ringer's solution having a reaction of pH 8.0. Basic dyes which gave the most definite results were safranin, Nile blue A, methylene blue, and methyl green. The epidermal cells of boron-deficient plants seldom absorbed dyes, but the collenchyma walls readily absorbed them regardless of the boron supply. Eosin was the only acid dye to penetrate the cells of the cortical parenchyma, and none of the basic dyes penetrated. None of the dyes penetrated the cells of the bundle sheath or of the cambium. The phloem elements absorbed basic more readily than acid dyes, though eosin was absorbed by the phloem of boron-deficient plants. The cells of the undifferentiated parenchyma and of the central pith appeared to be basic, since they readily absorbed the acid dyes Congo red and eosin. Tracheid walls absorbed both acid and basic dyes. Methyl green and safranin penetrated and stained the nuclei of the central pith without accumulating in the vacuoles or staining the cytoplasm.

The results of the various cytochemical tests are summarized in table 4.

Cytological Features of Sunflower Leaves. The leaves of sunflower plants are reliable indicators of boron deficiency. Initially they are pale green, then become mottled by unequal chlorosis; eventually the margins are inrolled and finally become necrotic. The veins are thickened, though none split open, as in the case of some plants.

Free-hand sections of living material of affected leaves were briefly submerged in the solutions hereafter named and transferred to slides for examination with the microscope. The protoplasmic components of the spongy parenchyma cells were greatly disorganized and their vacuoles were replete with aggregated material which readily absorbed neutral red. The palisade parenchyma cells were hypertrophied, their contents disorganized, plastids were scarce, and intercellular spaces absent. Their vacuoles contained varying amounts of phenolic compounds in the form of clumped granules (usually at one pole of the cell) or of vacuolate masses, both of which stained readily with neutral red. Lysis of the contents of those cells was common, resembling a type of proteolysis due to zinc deficiency previously described (Reed, 1938). The cell vacuoles were judged to have an acid reaction since neutral red penetrated slowly unless it was dissolved in Ringer's solution having a pH of 7.0 or 8.0, whereupon the dye entered rapidly and stained the vacuoles cherry red. Palisade cells which still possessed plastids were the last to be entered. Coacervates could be distinguished easily, since their external layer of phospholipid has an affinity for neutral red. They were also revealed in the palisade parenchyma of boron-deficient leaves when fresh sections were immersed in water on which a methylal-Sudan-III solution was superposed. The sections floated at the interface of the two liquids and absorbed Sudan III from the water-methylal phase. At the end of 30 minutes the sections were transferred to slides and examined with the microscope. The coacervates were discernible because the phospholipid layer was stained pink by the Sudan III. Coacervates of phenolic material were also demonstrated by the application to fresh sections of molybdenic reagent (freshly prepared), whereby the central sphere of

TABLE 4

Cytochemical Reactions of Sunflower Stem Tissues

Dye or reagent	Healthy plants				
	Epidermis	Cortical parenchyma	Phloem	Xylem	Pith parenchyma
Acidic dye	+	O	±	+	+
Basic dye	+	+	+	±	±
Reagents for oxidase	O	±	+	±	+
Reagents for reductase	O	O	+		O

Dye or reagent	Boron-deficient plants					
	Epidermis	Cortical parenchyma	Phloem	Undifferentiated parenchyma	Xylem	Pith parenchyma
Acidic dye	O	O	+	+	+	O
Basic dye	O	+	+	+	±	O
Reagents for oxidase	O	±	+	+	+	+
Reagents for reductase	O	O	+	±	O	+

+, positive.
±, weak.
O, negative.

phenolic compounds was rendered light brown and the phospholipid layer on the surface blue. The cytological evidence supports the idea of a non-compensated respiration in which the catechol substrate was irreversibly oxidized.

Incipient necrosis of phloem was plainly evident in sections of fixed material stained with phosphotungstic haematoxylin, but inorganic phosphates were not abundant in chlorenchyma tissue, when tested by the molybdenic reagent or by the Gomori method. This is similar to the condition found in the olive leaf. The contrast with the condition in the stems indicates that some activity peculiar to the leaf can block phosphatase activity. Further investigation of the interesting phenomenon is necessary.

Discussion of Sunflower Leaf Cytology. Everything learned about sunflower leaf cells supports the idea that the cell vacuole contains both the oxidizable phenolic compounds and an agent (polyphenol oxidase) which is able to catalyze their oxidation. In healthy, active cells the phenolic compounds are protected from irreversible oxidation by various hydrogen donors, such as ascorbic-dehydroascorbic acid or the dihydroxymaleic acid systems, to say nothing of thiol-containing groups such as cysteine and glutathione, the latter of which can serve as a reservoir of hydrogen for the cell. The balance in a healthy cell is such that the catechol particles (molecules or micelles) are randomly distributed in the vacuolar solution; but when nutrient deficiencies destroy the equilibrium between the hydrogenases and dehydrogenases, a desolvating action would occur and produce aggregates of quinones of the type observed in these cells. Wherever the quinones fail to be rehydrogenated, they will catalyze further oxidations, inhibit some links in the respiratory systems, and bring about death of the cells and formation of brown necrotic areas in the leaf. Where this rate is not so rapid as to outstrip the rate of reversible reaction, the cell or cells can survive. If the rate of quinone production is too rapid, the cells are killed. The strong activity of catechol oxidase could form positive charges on the surfaces of the phenolic precipitates which would attract the basic choline groups of the phosphatides and form an enveloping lipoidic layer which characterizes the coacervate. In other words, the formation of phenolic aggregates is due to a separation of phases caused by the desolvation of one of them.

SUMMARY

The unthrifty pathological condition of some plants may be due to the lack of one of those micronutrients formerly called "trace elements." Controlled experiments have shown that boron, although toxic in all but minute amounts, plays an important role in some of the most fundamental processes in plant cells. Characteristic deficiency symptoms appear when the amounts present or the supplying power of the soil falls below a minimum threshold value. The elucidation of certain physiological and cytological processes in relation to boron nutrition makes possible a better understanding of some fundamental problems in metabolism. None of the elements required for normal nutrition of plants acts singly, but in combination with many others; yet there are symptoms which are indicative of certain specific deficiencies. These are recognizable in the growth forms in leaf structure and in the quality of fruit production when other essential elements are not a limiting factor. Boron-deficient plants are dwarfed, and often the apical bud of the shoot dies. A deficiency of growth-promoting hormones has been assumed, but is not yet proved.

Boron-deficient olive trees grew slowly, and after a time the terminal buds of the shoots died; however, new shoots arose from lateral buds and made limited growth. Profound changes in the leaves were associated with a deficiency of boron; these included destruction of chlorophyll, irreversible oxidation of phenolic compounds which were polymerized to various relatively insoluble substances, and other factors which blocked the normal courses of metabolism. The bundle sheath and the sieve tubes of the phloem suffered derangements which blocked the normal conduits for the transportation of energy-rich materials in leaves.

Boron-deficient celery grew slowly. The petioles showed split veins and drying of the central tips. The leaflets were dwarfed, chlorotic, and eventually died at the margin. The roots died back from the tips. In severely affected plants, the apical buds of the shoots died and were succeeded by new shoots from accessory buds which succumbed after making a small amount of growth. Plants grown in nutrient solution containing 0.5 p.p.m. boron made healthy growths of tops and roots. It may be considered that the concentration of 0.5 p.p.m. boron is somewhere near the threshold requirement for boron in the case of celery.

The phloem region of boron-deficient petioles was the seat of high oxidase activity, and it was replete with black necrotic material. Eventually it was disrupted. Inorganic phosphates were trapped in the necrotic material in the disrupted phloem of boron-deficient plants. The parenchyma of healthy petioles maintained a lower oxidase activity than the same tissue in affected plants. Phenolic compounds in the celery seemed to escape irreversible oxidation, owing perhaps to the amounts of ascorbic acid which can act as a hydrogen donor. Melanotic substances, nevertheless, arose when the injury was severe in young shoots and roots, possibly from the oxidation of tyrosine or phenylalanine. The meristematic cells at the apex of the primordium were less affected by boron deficiency than the postmeristematic cells, in which hypoplasia and necrosis appeared early, followed soon by collapse of extensive areas. The primordia survived so long as sugars and amino acids could be translocated to them, but their existence was brief. The cells compensated in various ways for starvation—for example, hydration of biocolloids, abeyance of nucleoprotein synthesis, and accelerated respiration. The merismatic region of affected celery roots was killed early. The stele of the root had a high content of energy-rich phosphorus compounds, giving to its cells the ability to maintain a low oxidase level; hence the phosphate complexes might act as dehydrogenases. The cortical cells were richer in catechol and in catechol oxidase. Corresponding regions in healthy roots had a lower level of oxidase and phosphatase activity.

Radish plants showed clinical symptoms of boron deficiency by retardation of growth, chlorotic leaves, distorted roots which generally had black centers, and disorganized phloem in the leaf petioles.

Sunflower plants made extremely limited growth without boron, because of the death of the apical meristem and the chlorotic condition of the leaves. Necrosis of the apical bud preceded the formation of layers of undifferentiated parenchyma in the region between the xylem and phloem. Chlorosis of leaves and consequent impairment of photosynthetic activity was accompanied by desolvation of phenolic compounds and consequent impoverishment of the vacuolar solution.

The ratio of inorganic to total phosphorus in stem and leaves was highest in the boron-deficient plants, and correlatively they had a higher level of phosphatase and oxidase activity. Oxidase activity in the stems of boron-deficient plants was at a higher level than in healthy plants. The cells of leaves of affected plants showed many indications of disrupted metabolism, such as hypertrophy, vacuolar aggregates of the coacervate type, and finally, lysis.

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THE BRANCH WILT OF PERSIAN WALNUT TREES AND ITS CAUSE[1]

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INTRODUCTION

A BRANCH WILT DISEASE affecting Persian walnuts (*Juglans regia* L.) in California has been reported (Wilson, 1945, 1946).[3] The trouble was first noticed about ten years ago in the southern San Joaquin Valley; but, according to some growers, it has been present in the Sacramento Valley for an equal length of time. Within the past five or six years it has become a major disease of certain walnut varieties throughout the central valleys.

THE DISEASE

Name. Growers call the disease "wilt," "limb wilt," "twig wilt," or "branch wilt." The fourth name seems preferable, since the outer branches, not the limbs or twigs, are most commonly affected and since the disease is not a wilting of the entire tree, as the term "wilt" might imply.

Symptoms. As would be inferred from the name, the principal manifestation of the malady is the wilting of certain branches. During July and August the leaves wither, turn a deep brown, dry up (plate 1, *A*, page 443), but remain attached to the twigs. Because wilted leaves are very conspicuous against the surrounding green foliage, the disease is easily detected in an orchard during late summer. Only one other malady that might be confused with the branch wilt has been encountered. This is a twig blight[4] occurring in summer and suddenly withering the leaves on twigs; but it seldom if ever kills large branches in one season. Specimens of this other disease on small branches 1.0 to 1.5 inches in diameter were received from southern California; but in this instance evidently the disease first attacked a twig and then gradually extended to the branch, which it killed only after two or three years.

Once established in the outer branches, the branch wilt proceeds into the limbs (plate 1, *B*) and within a few years may involve most of the treetop.

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[3] See "Literature Cited" for citations, which are referred to in the text by author and date.

[4] The status of this disease is being investigated. A *Dothiorella* sp., which may be *D. gregaria*, the cause of the melaxuma canker on walnut branches, is found on the recently blighted twigs.

Linlithgow

When this occurs, not only are certain limbs killed outright, but others are so weakened by the death of large areas of bark and wood that they develop sparse sickly foliage and eventually succumb.

For some time after this disease appeared in Tulare County, growers mistook it for the effects of crown rot, caused by *Phytophthora cactorum*, which produces a general decline of the tree. But trees without evidence of crown rot developed branch-wilt symptoms, although, as was learned later, those with crown rot or with armillaria root rot (caused by *Armillaria mellea*) are more liable to attack by branch wilt than healthy, vigorous trees.

The bark on a branch affected by wilt is dead and discolored. The necrotic area commonly originates on the upper side of the branch, sometimes at a sunburned spot or around an injury made by the hooks or poles in harvesting (plate 2, *C*).

Wood underlying the diseased bark is dark gray to black (plate 2, *B*), and discoloration in the wood often extends up and down the branch well beyond the area of necrotic bark (plate 2, *D*). Elements of affected wood are filled with a dark brown substance, and the vessels with many tyloses.

A noticeable feature of diseased branches is the loosening and sloughing away of the periderm, exposing large areas of cortical tissue that are covered by a dark brown to black powdery deposit (plate 2, *A*). This deposit is composed of numerous dark brown fungus spores. So consistently are the loosening of the periderm and the presence of the sooty deposit associated with leaf-wilting symptoms, that these features serve to identify the disease even in winter, after the foliage has fallen.

Relation to Persian Walnut Varieties. Although the Franquette and Mayette varieties appear to be most subject to branch wilt, the Eureka is probably not much less susceptible. Others attacked are Payne, Meylan, Blackmer, and Bijou. The Concord, on the other hand, appears to possess some resistance, since in one orchard, trees of this variety were free of the disease though surrounded by badly affected trees of Franquette and Meylan.

The susceptibility of the Placentia and similar varieties is undetermined; few trees of these varieties are found in the districts where the disease is under observation.

Other Hosts. Although the native black walnuts *Juglans hindsii* Jepson and *J. californica* Wats. occur throughout California, and although the former, in particular, is used as a rootstock for Persian walnuts, only one wilted branch has been observed in these species. In one roadside planting near Winters, Yolo County, however, where wilt-affected Persian walnuts were interspersed between trees of *J. hindsii*, a few branches of the latter were found with the black spore deposit. These branches probably had been dead for a year, but the branch-wilt fungus was easily obtained in culture.

European chestnut trees, *Castanea sativa* Mill., about a mile northeast of wilt-affected walnut trees near Biggs, Butte County, developed typical branch-wilt symptoms in a few trees. Black spore deposits similar to those on walnut were found on these and on other branches that had died the summer before. The pathogenic and cultural characteristics of the fungus from chestnut are discussed later.

Wilting of the foliage in lemon trees (*Citrus limonia* Osb.) occurred in the Worth district, Tulare County, during the summer of 1946. According to the grower, the lemon trees were injured by freezing in 1938. The following year the more severely damaged branches were removed, but considerable areas of frost-injured bark remained in the tree. Wood rot, which developed in the cut-back limbs, probably caused the death of certain branches; but, for the most part, the wood of branches which died in 1946 was sound, though dry and discolored. A strip of bark along one side of the wilted branches had been dead for some time, presumably killed by freezing in 1938, but more recent necrosis of bark had occurred at the margins of these strips. Black layers of fungus spores were present beneath the periderm of recently killed bark, and gum exuded along the margins of the necrotic areas. Moreover, two branches with the diseased areas occurring only in tissue produced since the freeze, were found in one tree. A similar trouble was reported from this county in 1923 (Fawcett, 1936, p. 218) on frost-injured orange and grapefruit trees. Fawcett says of the disease: "Large branches are killed and dead bark-lesions also appear on the trunks, accompanied by

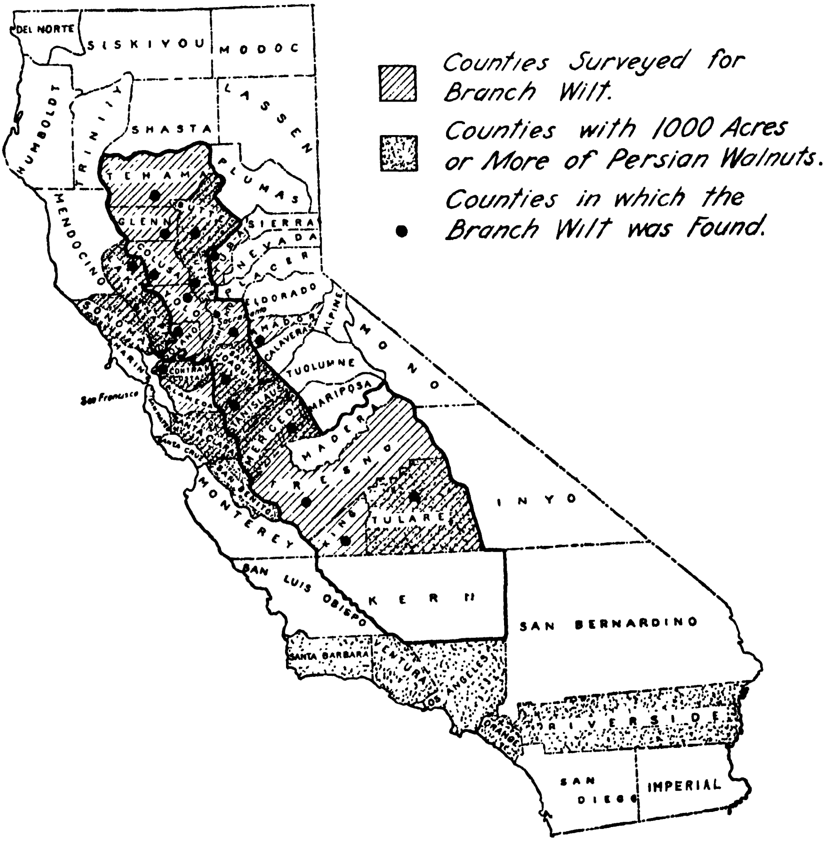

Fig. 1.—Map of California showing counties in which walnut branch wilt has been found.

much gum formation in large pockets under the bark." Fawcett also mentions the black spore deposits, but does not say in what manner nor at what time of year the branches died. The relation of this citrus disease to walnut branch wilt is considered in later sections.

Distribution in California. The map in figure 1 shows the major Persian-walnut-producing counties of the state. Walnut trees also occur in small groves or in roadside and border plantings in most agricultural districts. The Placentia and similar varieties are planted chiefly in the six southern counties, whereas Franquette, Mayette, and Payne are the chief varieties from Tulare County northward (Agricultural Adjustment Administration, 1936).

The area indicated by cross-hatching has been covered by the writer in a search for the branch wilt. Though the survey was not extended to the southern counties, agricultural representatives in that area were asked to send suspected material to our laboratory. Of the eight or more samples received, none proved to be branch wilt.

Within the area outlined on the map lie the San Joaquin and Sacramento valleys, where the search for the disease has been most intensive. Branch wilt is common in this area, occurring both in orchard plantings and on trees bordering roadsides and fields, but is most prevalent in Tulare County and along the Sacramento River in Colusa, Glenn, Butte, and Tehama counties. It also occurs on roadside trees in districts where walnuts are not grown commercially. On the other hand, the branch wilt was not found in important walnut districts of Sutter, San Joaquin, and Merced counties, though it is present in other sections of these counties.

Outside the interior valleys, the disease was found in Amador, Lake, and Contra Costa counties. Though of little consequence in the last county, in the other two it has done considerable damage to trees. No reports of the trouble were received from the coastal counties around San Francisco Bay, nor was it found by the writer on trips through Sonoma, Napa, Alameda, and Santa Clara counties.

Relation to Vigor of the Tree. Unthrifty trees are noticeably more subject to infection by branch wilt than vigorous ones. For example, as mentioned earlier, trees weakened by armillaria root rot or crown canker contract the disease more quickly and become involved by a more severe form of branch wilt than normal trees. In a certain orchard in Tehama County, eleven trees were sparse in foliage and obviously weak. At least three had been attacked by *Armillaria mellea,* while the rest bore symptoms of crown rot. During the 1945 season, branch wilt killed 88 branches in the weakened trees, but only 25 branches on a corresponding number of the surrounding vigorous trees.

Not uncommonly roadside trees which border unirrigated fields, and which therefore are subject to low soil moisture during the summer, are affected by branch wilt while near-by trees in irrigated orchards are free of the trouble. One case in particular involved a small orchard of Franquette trees that were visibly suffering from lack of soil moisture. Wilted branches were numerous in these trees, whereas in a well-cared-for Franquette orchard across the road to the east no branch wilt occurred.

Relation to Sunburning of Branches. A relatively high proportion of cankers appear around areas of bark that have been injured by exposure to the sun. Sunburning is believed to account for the fact that the incidence of the disease is sometimes two to three times greater on the south than on the north side of the tree.

Relation to Age of Tree. Young walnut orchards that have received proper care are usually less affected by branch wilt than older orchards. This seems to hold true of trees up to the age of ten or even fifteen years. On the other hand, certain young replants in affected orchards have manifested severe branch-wilt symptoms.

THE FUNGUS

Fungi Isolated from Affected Walnut Branches. Species of *Schizophyllum* and *Nectria* are sometimes isolated from branches that have been dead from wilt for a year or more, but these fungi are seldom found in recently wilted branches. A *Dothiorella* species is particularly common on blighted twigs, and its relation to the death of walnut branches is receiving attention. Apparently, however, this *Dothiorella* invades branches slowly and therefore does not cause the extensive symptoms typical of branch wilt.

The black spore deposit beneath the branch periderm usually is present when the branch wilts; hence the association of this fungus with the external symptoms of the disease was easily established. Moreover, according to results from about two hundred isolations from numerous specimens collected at various places, the dark-spored fungus was found in the bark and wood of over 90 per cent of recently wilted branches. Mycelium was found in most xylem elements, including the ray cells. Hyphae pass from one element to the other through the pits. Single strands of mycelium were seen to traverse five or six ray cells and then enter a vessel. This observation suggests that the fungus may enter the wood by way of the rays.

Inoculation of Walnut Trees with the Fungus from Affected Walnut Branches. Judging from the results of inoculating walnut trees over a period of years, the development of extensive symptoms by the dark-spored fungus depends on several factors, not all of which have been determined adequately. As shown in table 1, symptoms were not obtained when the fungus was inserted into slits in the bark[5] during January, February, March, or April; but necrosis of bark and wood resulted if inoculations were made in June and July. Much more extensive symptoms were obtained in the Mayette and Franquette varieties than in Concord. Young trees of the first two varieties were killed within 19 to 30 days after being inoculated in July with isolates of the fungus from two localities. Isolates from two orchards in a third locality also proved pathogenic when inoculated into older trees during July.

The sudden wilting of the foliage of inoculated trees resembled, in all respects, that on naturally infected branches. In several cases black spore masses were produced beneath the periderm a few days after the branches wilted, and the fungus was obtained in pure culture from the discolored wood 4 to 8 inches from the point of inoculation.

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[5] These slits were protected from drying by waxed paper over which adhesive tape was placed.

Characteristics on the Host and in Artificial Media. The fungus grows rapidly at 25°C or above on potato-dextrose, oatmeal, and malt-extract agar media, producing dark subsurface mycelium. At first the aerial mycelium is grayish, but later it becomes dark brown to black because numerous dark, profusely branched sporophores[6] are present. Although the formation of spores in culture is difficult to follow in detail, because sporophores break apart if disturbed, the process is believed to be as follows: An aerial hypha arising from the mycelium in the substrate branches several times. Cross-walls

TABLE 1

RESULTS OF INOCULATING WALNUT TREES WITH FOUR ISOLATES OF THE FUNGUS FROM WILTED WALNUT BRANCHES

Walnut variety, inoculation date, and orchard* where isolate was obtained	Length of necrotic areas, in inches, one month after inoculation		Remarks
	In bark	In wood	
Concord variety:			
March 13, 1942:			
Eyre orchard	0	0.1	Necrotic area in wood buried by new tissue
Newman orchard	0	0.4	Necrotic area in wood buried by new tissue
April 10, 1942:			
Eyre orchard	0	0.4	Necrotic area in wood buried by new tissue
Newman orchard	0	0.1	Necrotic area in wood buried by new tissue
June 23, 1942, Eyre orchard	2.5	3.0	Necrotic area in bark healed in summer
July 24, 1942:			
Eyre orchard	1.7	5.2	Necrotic area in bark healed in summer
Newman orchard	2.0	6.0	Necrotic area in bark healed in summer
July 3, 1943, Newman orchard	3.2	4.0	Sapwood killed 5 mm deep
July 3 1944, Newman orchard	2.7	5.0	Sapwood killed 3 mm deep
July 24, 1945, Hillman orchard	2.7	4.2	Sapwood killed 5 mm deep
Mayette variety:			
July 17, 1942:			
Eyre orchard	4.6	9.0	Sapwood killed 10 mm deep
Newman orchard	4.1	7.0	Sapwood killed 12 mm deep
July 24, 1945:			
Hillman orchard	7.3	7.0	Killed 2 young trees
Rowland orchard	9.0	14.0	Killed 1 young tree
July 30, 1945, Rowland orchard	12.0	15.2	Killed side branches of 3 trees
Franquette variety:			
July 17, 1942:			
Eyre orchard	4.3	10.0	One branch 0.5 inch diameter killed
Newman orchard	6.0	10.0	One branch 1.0 inch diameter killed
January 28, 1946, Rowland orchard	0	0	Inoculation wounds healed
February 28, 1946, Rowland orchard	0	0	Inoculation wounds healed
April 12, 1946, Rowland orchard	0	0	Inoculation wounds healed
May 21, 1946, Rowland orchard	0.5	0.7	Necrotic area in sapwood buried by new tissue
June 28, 1946, Rowland orchard	1.5	2.7	Healed around margins of necrotic area in bark
July 8, 1946 Rowland orchard	5.0	7.0	Killed 2 young trees
July 24, 1946, Rowland orchard	6.0	7.1	Killed 3 young trees

* Location of orchard from which isolates were obtained: Eyre and Newman orchards at Visalia, Hillman orchard at Biggs, and Rowland orchard at Los Molinos.

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[6] Conidiophore, if one uses this term to designate the entire fruiting structure. However, the stalk which bears the conidia is usually called the conidiophore. This structure is only slightly developed or absent in the fungus being discussed.

appear (fig. 2, *A*), first in the branches, then throughout the entire length of the sporophore. The hyphal segments become rounded, develop heavy walls and a dark brown color. Upon being disturbed, the entire sporophore will now break apart into one-celled and, in a few cases, two-celled segments. These segments (arthrospores) germinate readily when given the proper conditions. On potato-dextrose agar the fruiting structure is usually much longer and more profusely branched than that shown in figure 2, *A*.

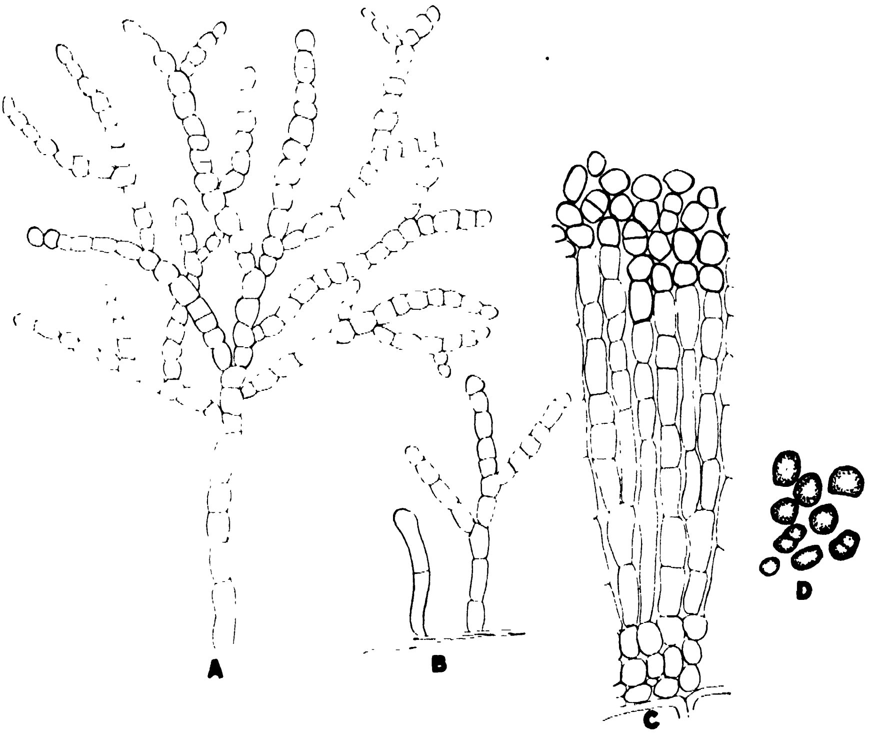

Fig. 2.—*A*, One of the simpler sporophores produced by the walnut branch-wilt fungus on potato-dextrose agar (× 525). *B*, Sporophores produced on diseased bark which was kept in a moist-chamber (× 400). The segments of the entire sporophore fall apart and function as conidia. *C*, Section through sporodochium produced under natural conditions (× 600). All cells of the upright hyphae arising from the slight hypostroma eventually mature into heavy-walled, dark-colored arthrospores. *D*, Arthrospores of various sizes and shapes from the host (× 600).

If thin radial sections of diseased bark are washed free of spores and mounted in a moist-cell, there soon develop short branched sporophores like those in figure 2, *B*.

The type of fruiting structure shown in figure 2, *A* and *B*, resembles that of a *Torula* Pers. and would place the fungus in this genus were it not that under natural conditions compact sporogenous bodies are commonly produced on the host. Upon examining the sooty deposit beneath the loosened periderm of branches, one often finds the spores in an almost unbroken layer, individual spores being separate from each other. Beneath this spore layer are a few segments, lighter colored and thinner walled than the spores. In other areas, however, the spore masses occur in clumps, at the base of which are a few thin-walled segments. These clumps are individual fruiting structures that have almost completely broken up into arthrospores. If the periderm of recently wilted branches is removed carefully, immature fruiting structures often can be found. Plate 3, *D* and *F*, shows mature and immature structures, 1 mm or more in diameter, which were produced on a small walnut branch. When not too numerous these often raise the periderm into small pimples (plate 4, *A*). Plate 3, *A*, is a longitudinal radial section through the bark cortex, and through a partly mature fruiting body. At this stage the structure (fig. 2, *C*) is composed of a few thin-walled basal cells (hypostroma) from which arise parallel hyphae in a compact mass. The hyphae are septate throughout their length, and some of the end segments have become thick-walled, dark, and separate from each other. Apparently segmentation of hyphae progresses downward from the ends (basipetally), until eventually the entire body, except the hypostroma, crumbles into a mass of separate spores. When numerous, the crumbled fruiting structures produce an unbroken layer over a considerable area of cortex.

TABLE 2

PATHOGENICITY OF ISOLATES FROM PERSIAN WALNUT, EUROPEAN CHESTNUT, AND GRAPEFRUIT TO YOUNG TREES OF THE FRANQUETTE VARIETY OF PERSIAN WALNUT

Isolated from	Inoculated July 8, observed July 27		Inoculated July 24, observed August 17	
	Range of canker lengths on trunk, inches	Effect on trees	Range of canker lengths on trunk, inches	Effect on trees
Walnut, Los Molinos ..	4-6	One side branch wilted*	4-5	Several side branches wilted*
Chestnut, Biggs .	6-8	Entire tree wilted	5-6	Several side branches wilted*
Grapefruit, El Centro..	3-5	One side branch wilted*	4-5	Several side branches wilted*

* Certain trees later died.

On cornmeal agar[7] and inoculated steam-sterilized walnut twigs, the toruloid type of fruiting structure is sparse; instead, an elaborate stroma develops (plate 3, *B*). The arthrospores are produced by segmentation of hyphae arising from the surface of this body.

In one culture series on cornmeal agar, 1 to 3 spheroid cavities developed in certain of the fruiting structures. A number of the cavities were filled with spindle-shaped to ovoid bodies resembling pycnidiospores. Unfortunately, none of the bodies matured, and none of the pycnidiumlike cavities has since been observed in single-spore cultures. Some time later, well-developed stromata containing flask-shaped cavities with ostioles were found under and among the arthrospores of the wilt fungus on one small walnut twig. Though most of the cavities were empty, a few contained two or three ovoid, hyaline spores, which were produced on short stalks arising from the sides of the cavity. Because these spores were so scarce, they could not be successfully segregated from the numerous arthrospores surrounding the stroma. Careful search has not revealed any further cases of this sort; and since a *Dothiorella* species, which produces a similar structure, is not uncommon on dead walnut twigs, possibly the stromata may have been those of such a fungus. Decisions on the significance of these observations must therefore be postponed.

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[7] Shear's formula: 20 grams of cornmeal per liter of water, heated in water bath at 58°C for 1 hour and filtered. To filtrate was added 15 grams per liter of agar.

A few ascomycetes found growing on wilt-infected walnut limbs have not proved to be the sexual stage of the branch-wilt fungus. Because no perfect stage has been found and the status of the pycnidial stage is doubtful, the branch-wilt fungus must be classified on the basis of its conidial fruiting structure.

The first question concerns the nature of this fruiting structure. Both on the host and in young cornmeal-agar cultures, the fertile hyphae arise from what appears to be a loosely packed mass of mycelium, though the elements of the mycelium may eventually lose their identity and, in the structures on agar, sometimes form a compact hypostroma (plate 3, *B*). According to a definition by Clements and Shear (1931, p. 196) this structure is a sporodochium, which "is to be regarded as a compacting of hyphae and conidiophores rather than a new development from a reduced fruiting-body with short or obsolescent basidia."

Before further identification is attempted, the results of comparing the fungi from the various hosts must be given.

COMPARISONS OF ISOLATES FROM CHESTNUT, CITRUS, AND WALNUT

Upon being shown the walnut pathogen, H. S. Fawcett of the University Citrus Experiment Station at Riverside recognized it as resembling a fungus he obtained in 1923 from frost-injured orange and grapefruit trees in Tulare County, California. According to him (Fawcett, 1936), the fungus appeared to be *Torula dimidiata* Penz. His description of the black powdery layer of spores under the branch periderm is reminiscent of the manner in which the walnut pathogen sporulates. Mr. Fawcett kindly furnished the writer with his 1923 specimens, and later sent cultures and specimens obtained from grapefruit trees at El Centro, California, in 1945. As reported earlier in this paper, the writer found a similar fungus on wilted lemon branches in Tulare County in August, 1946. Black powdery layers of spores occurred at various places beneath the periderm of affected branches, and the fungus was readily isolated from the wood of recently wilted branches.

In the following sections are reported comparative studies of the isolates obtained from grapefruit at El Centro, chestnut at Biggs, and Persian walnut at Los Molinos. The black-walnut isolate was obtained too late to be included in certain of these studies.

Pathogenicity to Persian Walnut. In the summer of 1946 three series of inoculations were made on young Franquette (Lattin strain) walnut trees growing at Davis. The first, June 6, resulted in the formation of small cankers (1 to 2 inches long) by all three isolates, those produced by the walnut and chestnut isolates being somewhat longer than those from the grapefruit. In the next two series of inoculations (made in July), however, extensive symptoms were produced by all three isolates (table 2). On the whole, the chestnut isolate appeared to be the most active, completely involving the bark and wood for several inches around the point of inoculation and killing the young trees within 19 days. Certain trees, however, were later killed by the walnut

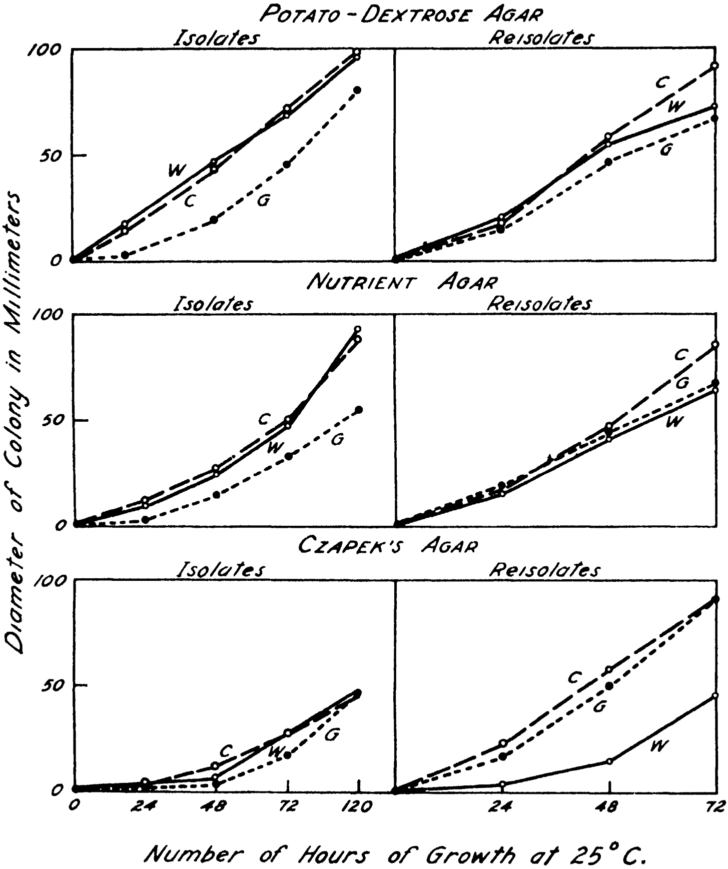

Fig. 3.—Rate of growth of the (W) walnut, (C) chestnut, and (G) grapefruit isolates on three solid media.

and grapefruit isolates. Judging from these tests, there seemed to be little doubt that the grapefruit and chestnut isolates were able to cause symptoms identical with those produced by the walnut pathogen. All three isolates were recovered from the wood of inoculated trees in areas 4 to 6 inches from the point of inoculation. Single-spore cultures of the reisolates were kept for comparison with the original isolates. These comparisons are described later.

Growth on Various Media. The three isolates were grown on several types of solid media. The principal difference in growth on most media was the tendency for the grapefruit fungus from El Centro to develop the shorter and sparser aerial hyphae. All produce the toruloid type of conidiophores on potato-dextrose, nutrient, malt-extract, and oatmeal agars.

Of single-spore cultures of the original isolates, the one from grapefruit consistently grew slowest on potato-dextrose, nutrient, and Czapek agars (fig. 3). This difference was less noticeable, however, after passage of the isolates through walnut trees (fig. 3, reisolates); apparently, the growth rate was modified by the host.

Size and Shape of Arthrospores. Mature arthrospores of all three isolates varied widely in size and shape. The shape of arthrospores from walnut, chestnut, grapefruit, and lemon bark was similar. The color was the same in hue, but slightly different in shade, those from citrus hosts being lighter. On cornmeal agar the grapefruit and lemon isolates produced spores that were preponderantly spheroidal, whereas on this medium the isolates from chestnut and walnut produced spores more irregular in shape, some being spheroidal, others ovoidal or roughly cylindrical. It was learned later, however, that among single-spore isolates of the fungus from Persian walnut, some produced a greater number of spheroidal spores than others. Since these differences in spore characteristics are associated with differences in the structure of the sporodochia, the subject is discussed under another heading.

To obtain a single expression for size, the maximum and minimum dimensions of each spore were determined. These values, averaged as the mean diameter, are as follows:

Isolate	Mean diameter of spores, in microns	
	From diseased bark	From potato-dextrose agar
Walnut	5.5	5.1
Chestnut	5.1	5.5
Grapefruit	5 2	4 5

Though the size of spores from the host did not differ greatly among the three isolates, spores of the grapefruit isolate taken from potato-dextrose agar were somewhat smaller than those of the other two. When, moreover, frequency curves were plotted for the average mean diameter of 100 spores of each isolate, spores of the walnut and chestnut isolates were found to be in the same modal class, whereas those of the grapefruit isolate were one class lower. This held true for spores from the host as well as those from culture. Apparently, therefore, the grapefruit isolate tends to produce the smallest spores.

Temperature Requirements. The walnut pathogen, in several tests, required fairly high temperature for growth. At 16°C and below, it grew slowly; but the rate increased rapidly with increases in temperature above 16°, the most rapid mycelial growth occurring between 30° and 33°. Above 33°, however, growth was slower.

According to the data in figure 4, all three isolates responded to temperature in about the same way, although here, as in tests reported in the previous section, at all temperatures the grapefruit isolate grew somewhat more slowly than the other two.

Production of Sporodochia. On grapefruit bark collected by H. S. Fawcett and on lemon bark collected by the writer, the spores frequently occurred in clumps scattered over the surface of the cortex. These clumps were found to contain varying amounts of stromatic tissue with an overlying layer of spores. These spores had become separated from one another, and their original arrangement lost. Since immature fruiting bodies were not found on lemon or grapefruit, as they were on walnut, the manner in which the spores were formed was not determined on these hosts. The mature fruiting structures on citrus, chestnut, and walnut, were similar in all respects. Plate 4, *B*, *C*, and *D* (page 436), shows the nature of the spore layers on these hosts.

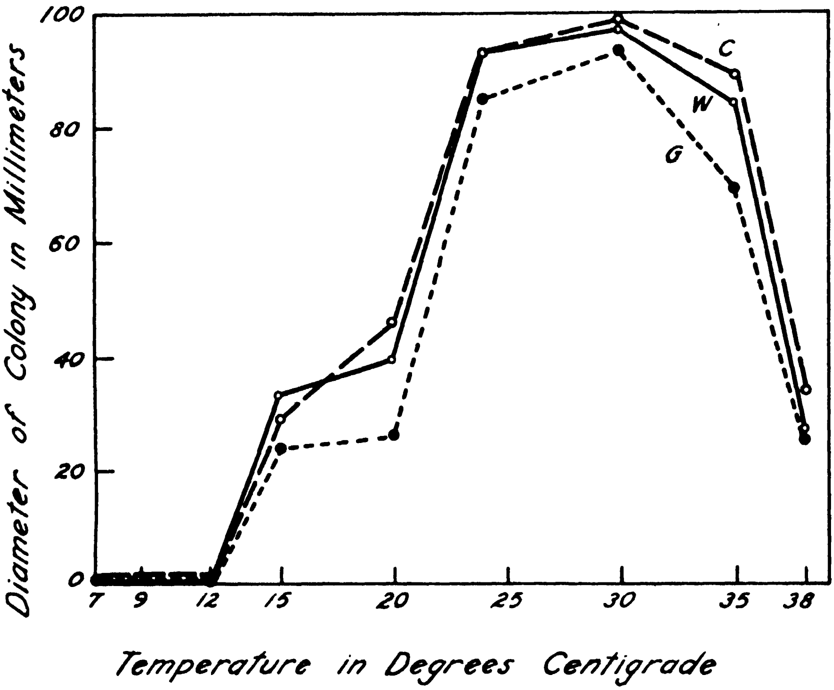

Fig. 4.—Relation of temperature to mycelial growth of the (*W*) walnut, (*C*) chestnut, and (*G*) grapefruit isolates.

Sporodochia produced by the Persian walnut, chestnut, and grapefruit isolates on inoculated steam-sterilized walnut twigs are pictured in plate 3, *E* (page 435).

As noted earlier, isolates from Persian walnut produced more or less densely packed sporodochia on cornmeal agar. In some cases (plate 3, *B*) these bodies contained fairly large amounts of stromatic tissue, from which arose the sporogenous hyphae. In other cases, however, the individual hyphal elements of the sporodochia retained their identity and were grouped together in a loosely compacted body with little or no hypostroma.

For comparing sporodochial production, the walnut, chestnut, and grapefruit isolates, and the reisolates, mentioned in foregoing tests, together with six other single-spore isolates from Persian walnut, one from black walnut, and one from lemon, were grown on cornmeal agar at 25°C. Though differences in the rate of growth and in production of sporodochia occurred, these features varied no more between the isolates from the three hosts than between those from Persian walnut. The grapefruit and lemon isolates produced spores that were preponderantly spheroidal, a fact mentioned earlier. These isolates also developed sporodochia that were more compact and contained greater amounts of hypostroma than sporodochia of certain walnut isolates. On the other hand, two of the walnut isolates produced compact sporodochia, though few in number. The grapefruit reisolate differed from all others in producing no spores on this medium, though it sporulated freely on potato-dextrose agar. Apparently, therefore, the rather wide differences in the structure of the sporodochium and shape of spores which occurred on cornmeal agar did not serve to distinguish the grapefruit isolate from the walnut and chestnut isolates.

IDENTITY OF THE WALNUT PATHOGEN

Judging from the foregoing comparisons, the fungus from citrus, though differing from the walnut pathogen in size of spores and in abundance and length of aerial growth on solid media, is similar in other characteristics; so far as these studies go, there seems to be no reason for considering it a separate species. Moreover, there appeared to be no major difference between the chestnut and the walnut isolates.

The writer is uncertain how a reclassification of the citrus fungus should affect the status of *Torula dimidiata*. Unquestionably, the fungus from citrus, chestnut, and walnut resembles Penzig's organism, producing under some conditions single toruloid conidiophores; but, as we have seen, sporodochia are produced on the host. The sporodochium, except for the thin hypostroma, breaks apart into a mass of arthrospores. The bark periderm must contribute to this process, for at most the sporodochium is a loosely compacted structure and, being compressed between the periderm and cortex, is crushed into a flattened disorganized layer as the conidial chains mature. From this mass one might conclude that the spores had been produced on numerous separate conidiophores, or at most on small tufts of conidiophores. One might obtain support of this conclusion by washing the old spores from the cortex and keeping the bark in a moist chamber. Under such conditions, as we have seen (fig. 2, *B*), short toruloid sporophores develop.

Penzig (Penzig, 1887) reported his fungus as producing irregular, dark brown, velvety tufts on lemon bark. The arthrospores are said to measure 4 to 5 μ—slightly (but possibly not significantly) smaller than those of Fawcett's organism. Except in this and in other small details, Fawcett's organism fits Penzig's description, provided the sporophores that arise from the periderm-free cortex of bark kept under moist conditions are considered to be the typical fruiting structure. That this typical structure is a sporodochium, not a single sporophore, can hardly be disputed if early stages of fructification are studied. Although Penzig possibly dealt with the fungus described herein, so that his may have been the first description, this description is very inadequate. Since it is possible that he had another fungus, the writer is not willing at this time to alter the status of *Torula dimidiata*.

The possession of sessile sporodochia, dark brown mycelium, and conidia of the same color would place the branch-wilt fungus in the family *Tubercu-*

lariaceae, section *Dematieae.* Though the arthrospores are sometimes two-celled, because an occasional septum fails to reach the stage where separation occurs, the great majority of these bodies are one-celled. Apparently the sporodochium is without spines or setae, and the conidiophore is much reduced, if not absent. The arthrospores are smooth and, being formed by the segmentation of hyphae, are of course catenulate. These characteristics apparently place the fungus in the genus *Exosporina* Oudemans, whose form species is *Exosporina laricis* Oud.

According to Oudemans (Oudemans, 1904), Corda and Saccardo had described as *Trimmatostroma salicis,* a similar fungus on willow. Since, however, the genus *Trimmatostroma* as constituted by Corda produces multicellular conidia, Oudemans erected the genus *Exosporina,* which he described as including exposed or endogenous fungi with no "stroma" (hypostroma) or with this structure slightly developed. Conidia are arranged in chains, separating individually, homomorphic, continuous, and colored. Concerning *E. laricis* he says that the fruiting structures are amphigenous (presumably with regard to their occurrence on both sides of larch needles), exposed, punctiform, and black. The rather long "stipitate" chains are closely condensed into a convex "placenta." The conidia are at first angular, then globular, ferruginous, continuous, 5 to 6 x 5 μ, and separating individually.

The branch-wilt fungus resembles the type species in shape of conidia, which are first angular, then globular; and in the manner in which the conidial chains separate into segments. In size the conidia of the branch-wilt fungus vary somewhat more than those of *Exosporina laricis,* the former in one series of measurements being 4.2 to 8.3 μ long by 3.3 to 8.3 μ wide (average 6.1 x 5.2 μ). Judging from Oudemans' illustrations, which are somewhat diagrammatical, the two fungi differ with respect to the hypostroma and the branching of the sporodochial chains. Whereas Oudemans shows *E. laricis* without hypostroma and with nonbranched conidial chains, the sporodochium of the branch-wilt fungus possesses a definite hypostroma and the conidial chains are not infrequently branched. Apparently, moreover, *E. laricis* is not found on the woody parts of the host but is restricted to the needles, whereas the branch-wilt fungus grows extensively through the wood and bark tissues. These contrasting features would seem to indicate the two fungi though similar are not identical.

Newodowski (1912) reported *Exosporina mali* Newodowski as pathogenic to young apple branches in Tiflis. The conidia of this fungus are 11.0 to 25.0 μ by 6.0 to 8.5 μ, much larger than those of the branch-wilt pathogen. The terminal spores on the conidial chains of *E. mali* possess apical protuberances, a feature not found on spores of the branch-wilt fungus.

Hendersonula toruloidea Nattrass, found on peach twigs in Egypt and citrus twigs in Cyprus (Nattrass, 1933), produces toruloid conidiophores resembling to some extent those developed by the branch-wilt pathogen on potato-dextrose agar. On the host the conidiophores of *H. toruloidea* are said to arise from a thin layer of fungal tissue which later develops into a stroma containing pycnidia at different levels. The pycnidiospores, so long as they remain inside the pycnidium, are colorless and nonseptate, but upon being extruded develop two septa and a dark-colored central spore. In 1931 A. J.

Olson (Fawcett, 1936) found a similar fungus on frost-injured citrus branches near Hamilton City, Glenn County, and later proved this fungus capable of producing wood rot and fruit rot of oranges and lemons. In a recent letter to the writer, Mr. Fawcett says that he noted similarities between this fungus and the fungus he regarded as *Torula dimidiata,* but since he had no cultures of the latter was unable to compare the two.

The writer found no pycnidia associated with the branch-wilt fungus on citrus. As noted earlier, stromata containing pycnidia and a few pycnidiospores were present on one small walnut branch among the sporodochial stage of the branch-wilt fungus, but no cultures were obtained from these spores. The pycnidia, however, were borne at approximately the same level, and all pycnidiospores seen, whether inside or outside the pycnidium, were hyaline. Judging from these two features, therefore, the stromata resembled those of a *Dothiorella* more closely than they did those of *Hendersonula toruloidea.*

Apparently, therefore, *Torula dimidiata, Hendersonula toruloidea,* and the fungus described here are similar in certain morphological features. *H. toruloidea* and the branch-wilt fungus, moreover, possess similar temperature requirements. On the basis of the available information, however, one is not justified in regarding them as identical.

The name *Exosporina fawcetti* n. sp. is proposed for the fungus on Persian walnut, European chestnut, and citrus.

Exosporina fawcetti n. sp. A brown to sooty layer of spores develops beneath the periderm of diseased branches, causing this layer to separate from the underlying cortex. Numerous pulvinate, sessile, dark sporodochia up to 400 μ in diameter and 150 μ high develop over surface of the cortex. Sporodochium consists of compact, parallel, occasionally branched, chains of conidia arising from a slight hypostroma. Conidia (arthrospores) in chains mature basipetally, separate readily, are 1-celled (rarely 2- to 3-celled), globoid to slightly angular, dark brown, variable in size—3–8 μ (averaging 5.5 μ); the mycelium is dark brown. Aerial hyphae adjacent to sporodochia, torulose, dark brown, separating into great numbers of cells or fragments.

Habitat: On branches of Persian walnut, *Juglans regia;* European chestnut, *Castanea sativa;* lemon, *Citrus limonia;* grapefruit, *C. grandis;* and orange, *C. sinensis,* in California. Type specimens of the fungus deposited in the Mycological Collections of the Division of Botany, University of California, Berkeley. Cotype specimens in the Mycological Herbarium of the United States Department of Agriculture, Beltsville, Maryland.

Exosporina fawcetti n. sp.: Stratum brunneum vel usque fuligeneum sub peridermio evolvet, efficiens ut peridermium de cortice secedat. Numerosa sporodochia pulvinata, sessilia, fusca usque ad 400 μ diametro et 150 μ altitudine supra corticem evolvent. Sporodochium constat ex conidiorum catenis parallelis, de hypostromate parum evoluto surgentibus, aliquando ramificatis. Catenae conidiorum basipetaliter maturescunt, fragmentis facile secedentibus. Sunt conidia continua, raro duarum vel trium cellularum, globosa vel leviter angulata, fusca, magnitudine varia, 3–8 μ (5.5 μ); mycelio fusco; hyphis adfinibus sporodochiis torulosis, fuscis, ad numerosa segmenta secedentibus.

Habitat in ramulis juglandis regiae, castaneae sativae, citri limoniae, citri grandis et citri sinensis, in California.

DISCUSSION

Certain relations apparently exist between the incidence of the disease and one or more environmental factors. The first relation concerns the occurrence of branch wilt in all parts of the interior valleys and in adjacent foothill districts where summer temperatures are high. In those places, the malady occurs even where there are few Persian walnut trees. On the other hand, branch wilt, in a severe form at least, is not found among the extensive plantings of susceptible varieties in coastal districts, where summer temperatures are much lower than in the interior. Since the height of disease activity comes in the summer, and since the fungus grows best at comparatively high temperatures, a correlation seems to exist between temperature and disease severity.

The second relation concerns the high degree of selectivity which branch wilt exhibits in its attack on susceptible hosts. A correlation between severity of infection and vigor of the trees has been cited. It is not apparent, however, whether the pathogen finds conditions for development more favorable in weakened than in normal branches or whether the sparsity of foliage that accompanies low vigor contributes to sunburning of the branches and consequently to an increase in opportunities for infection.

Branch wilt shows a selectivity with regard to trees of different ages: the incidence of infection is noticeably low in solid plantings of young trees. Young replants in older orchards where branch wilt is present, on the other hand, sometimes contract the disease severely. Judging from the inoculation tests, young trees are not noticeably resistant to the development of branch wilt, once the fungus gains entrance to the bark. One might suppose, therefore, that young orchards are free of the disease only because the trees are farther away from infection centers. Since, however, the fungus seems ideally adapted to wind dissemination because of an abundant supply of dry powdery spores, the freedom of young orchards from wilt probably is due to some other cause or causes, such as freedom from bark injuries and sunburning.

The earliest cases of walnut branch wilt were found in Tulare County, where the disease has since gained its greatest headway. There is evidence, however, that it appeared about the same time in certain parts of the Sacramento Valley. Here, too, it increased greatly in subsequent years. These facts might indicate that the disease either is of recent origin, has been introduced from another region, or has spread to Persian walnuts from another host. Nothing, however, has been learned that would support the theory of introduction, and little to suggest that the disease spread from another host. Though the black walnuts would be the first host suspected, search has revealed few black-walnut branches with the fungus. The chestnut is excluded as a likely source of infection because no chestnut trees are grown in districts where the disease has become most prevalent. We know, of course, that the fungus occurred on citrus near Porterville twenty-three years ago, and is present near there today in at least one lemon grove. The Porterville district is about 20 miles south of the center of walnut-wilt infection in Tulare County. Though few large walnut plantings occur near Porterville or in areas immediately north, one large planting is located about 5 miles west. In this orchard, wilt developed on a few branches, apparently for the first time, in 1946. Moreover, except in a small orchard at Exeter, the disease is of little consequence a few miles south of the principal center of walnut infection, which is about 5 miles west of Visalia. These circumstances hardly suggest that citrus was the host from which the fungus spread to walnut. More evidence is needed before conclusions can be reached on the origin of this disease.

SUMMARY

A disease affecting Persian walnut trees, *Juglans regia,* and manifested as a sudden wilting of the leaves on certain branches, is described. The bark and wood of affected branches are dead and discolored. A dense layer of fungus spores develops between the cortex and periderm of the branch. Large areas of the latter structure loosen and slough away.

Franquette and Mayette are the varieties chiefly affected by branch wilt, though the Payne, Eureka, Meylan, Blackmer, and Bijou are also attacked. The Concord appears to possess some resistance.

A few branches of northern California black walnut, *Juglans hindsii,* were found with the black spore deposit; but noticeable wilting of the branches was not found extensively in this species nor in *J. californica.* European chestnut, *Castanea sativa,* is attacked by the fungus, the wilting of branches being similar to that in Persian walnut. Wilting of the branches of lemon, *Citrus limonia,* was observed in one orchard in Tulare County. A similar disease was found on grapefruit and oranges in that county in 1923 by H. S. Fawcett, who reported that the fungus present resembled *Torula dimidiata* Penz. He also found it on grapefruit near El Centro, Imperial County, in 1945.

Branch wilt is found in walnut trees throughout the Sacramento and San Joaquin valleys and adjacent foothill areas, but has not been reported from the central coastal districts, nor from the principal walnut regions in southern California.

Unthrifty trees are noticeably more subject to wilt than vigorous ones. Trees affected by crown rot or armillaria root rot were shown to suffer more than normal trees.

Branches with bark injured by exposure to the sun are commonly attacked.

Young walnut trees, except replants in mature orchards, are less subject to branch wilt than older trees.

The dark-spored fungus that produces the black layer of spores beneath the branch periderm was found in the wood of a majority of recently wilted branches. Inoculated into Persian walnut trees, this fungus produced extensive cankers that girdled and killed the branches.

On many cultural media the pathogen produces numerous branched sporophores arising singly or in small groups from the substrate. As the sporophores mature they become septate throughout their length, the segments developing thick walls and a dark brown color. At this stage the segments easily separate into one-celled bodies (arthrospores), which will germinate under proper conditions. Under natural conditions on the host, however, sessile pulvinate sporodochia are produced. The spores are produced by a basipetally progressive segmentation of closely packed, parallel hyphae arising from a slight hypostroma. There was no conclusive evidence that the fungus produces other spore forms.

Although the type of fruiting structure produced in most media would place the fungus in the genus *Torula* Persoon, the development of sporodochia would assign it to the genus *Exosporina* Oudemans.

So far as the morphological and cultural characteristics were studied, the fungus from diseased grapefruit branches differed only in minor characters from the walnut pathogen, and the fungus from chestnut not at all. The grapefruit and chestnut isolates, moreover, produced symptoms in Persian walnut similar in all respects to those produced by the walnut pathogen. The fungus from citrus is therefore regarded as identical with that from chestnut and walnut. The name *Exosporina fawcetti* n. sp. is proposed.

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PLATES

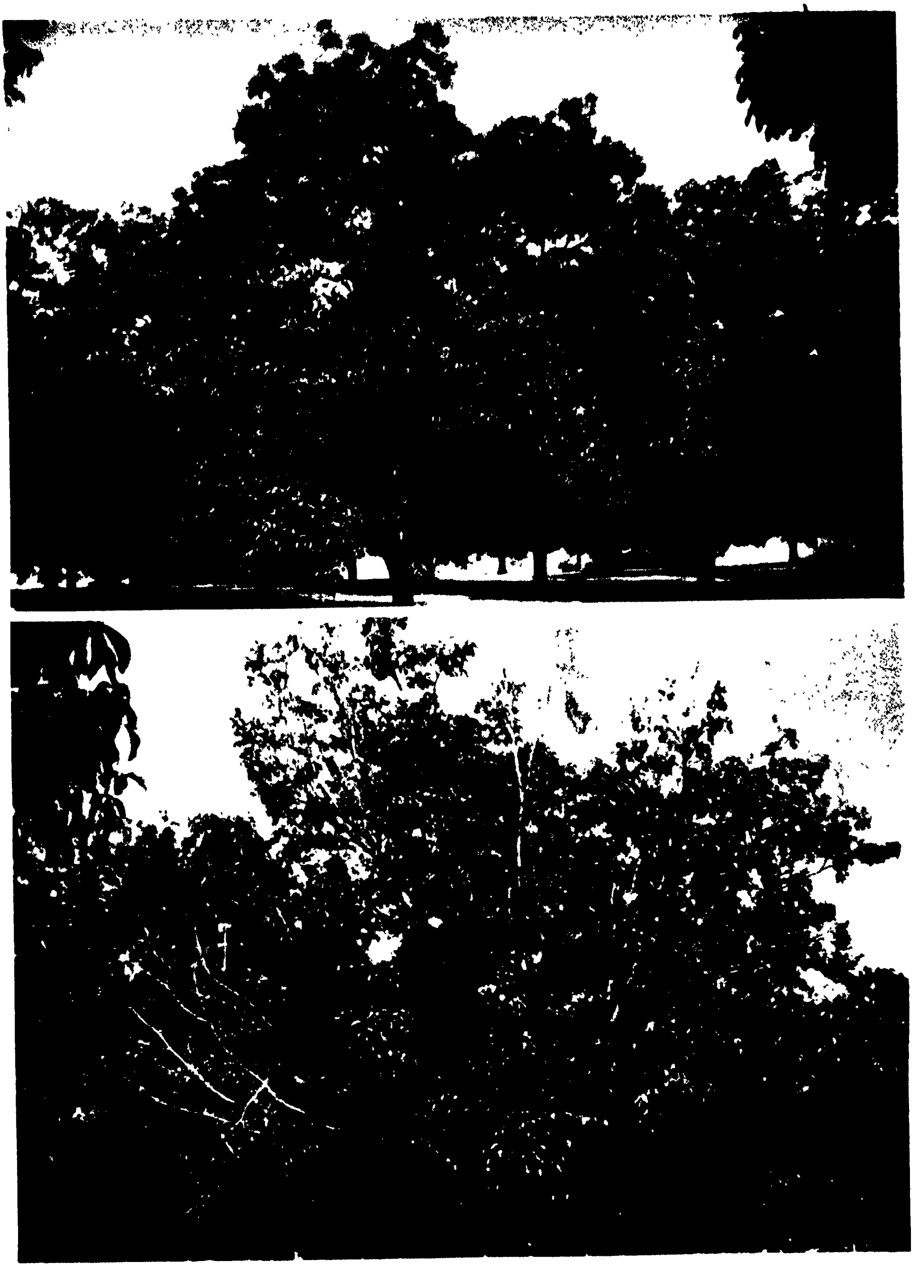

Plate 1.—Persian walnut trees affected by branch wilt.
(Photographed by E. F. Serr.)

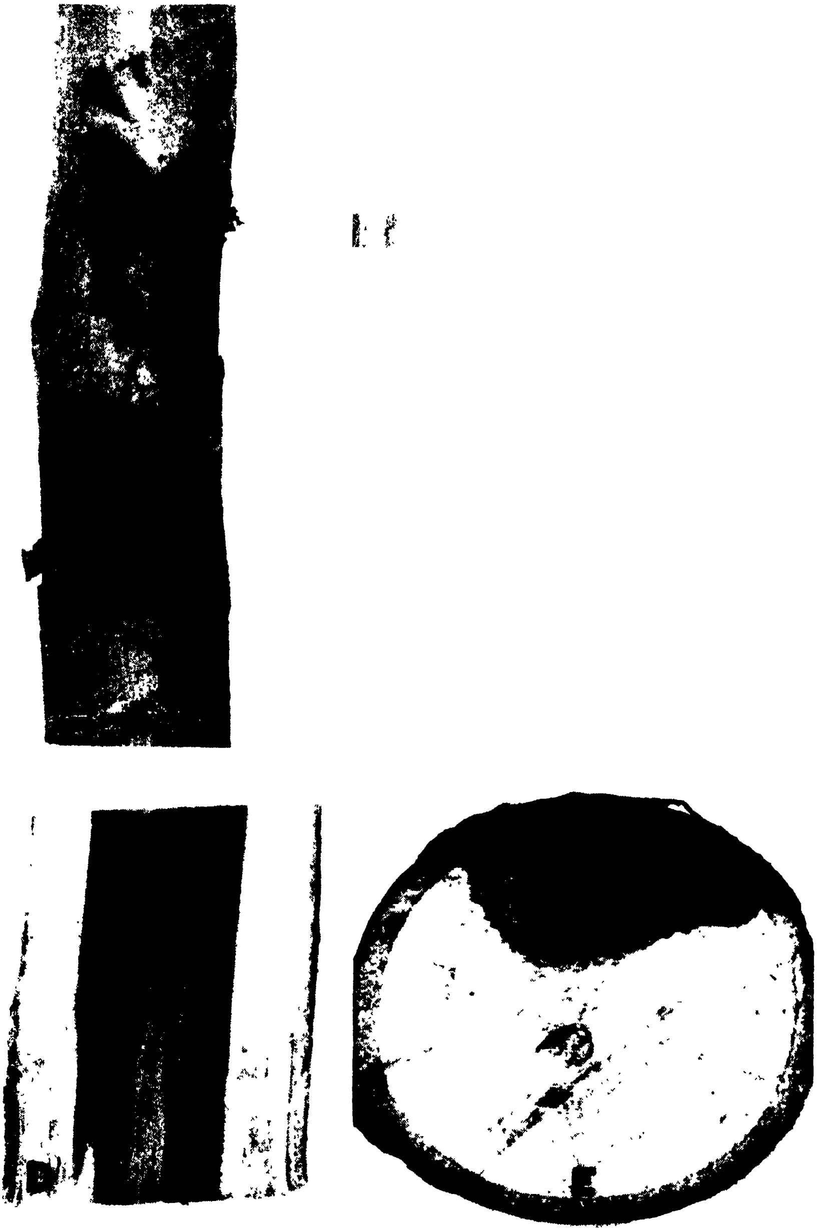

Plate 2.—The bark and wood of wilt-affected walnut branches: *A*, periderm sloughed away exposing the cortex, which is covered by a sooty layer of fungus spores; *B*, grayish discoloration of the wood in a branch recently killed by branch wilt; *C*, a wound, probably made by a harvesting hook, surrounded by a branch-wilt canker; *D*, black discoloration extending through the heartwood of a diseased limb; *E*, a diseased area extending into the wood from one side of a branch.

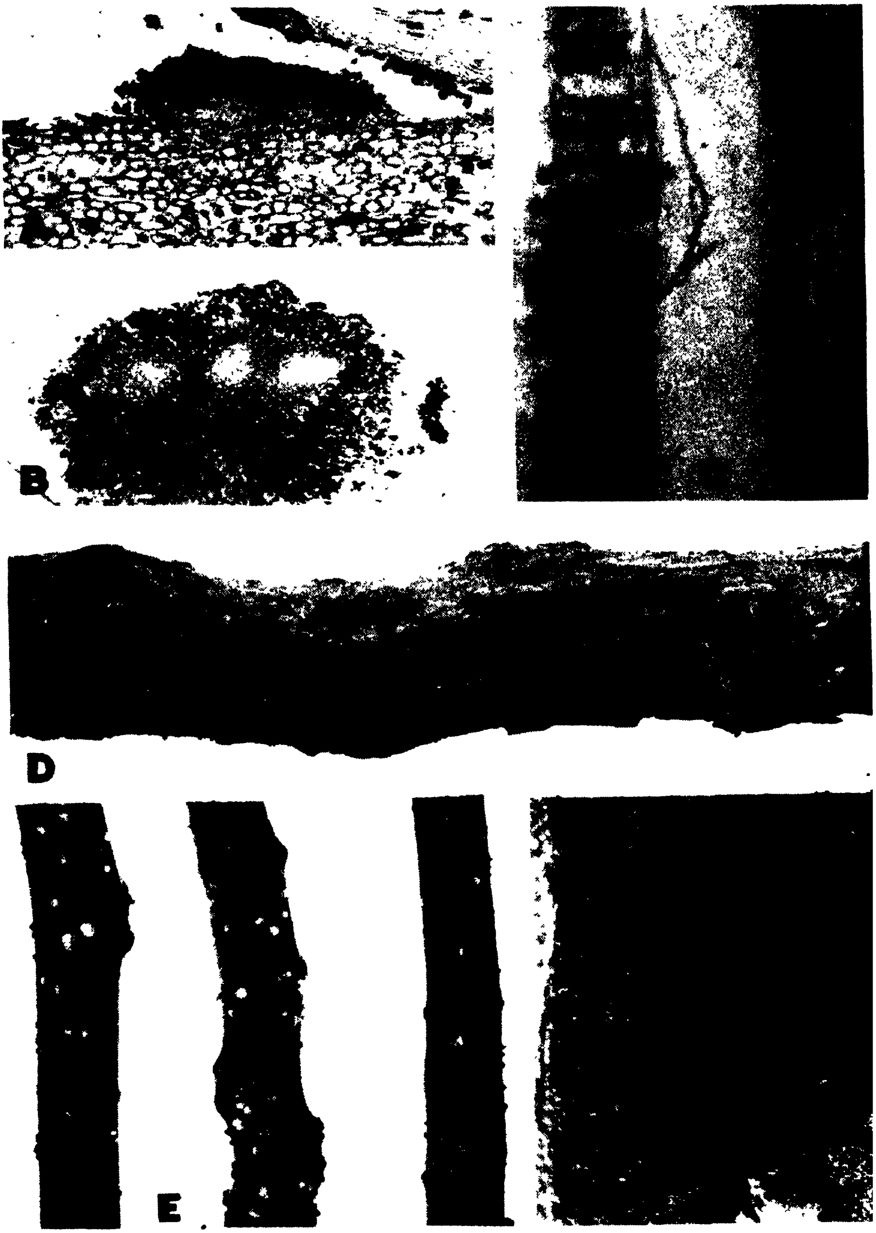

Plate 3.—*A*, A sporodochium of the branch-wilt fungus on the cortex of diseased walnut bark (longitudinal section, × 80). The loosened periderm is visible above the sporodochium. *B*, A sporodochium produced on cornmeal agar (longitudinal section, × 80). *C*, Mycelium of the branch-wilt pathogen in a vessel (× 225). *D* and *F*, Immature and mature sporodochia on the cortex of branches (*D*, × 2; *F*, × 5). *E*, From left to right, sporodochia produced by the walnut, chestnut, and grapefruit isolates on artificially inoculated, steam-sterilized walnut twigs.

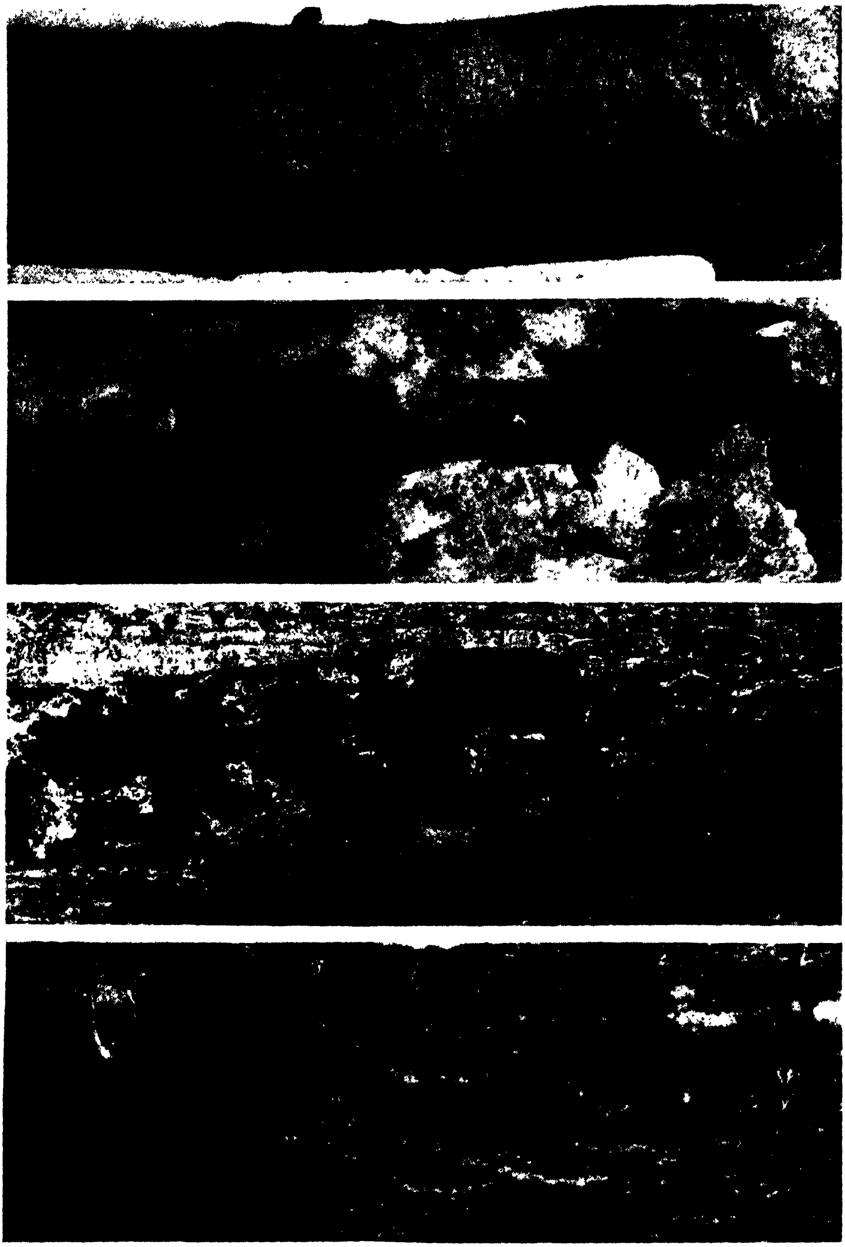

Plate 4.—*A*, Raising of the periderm of a walnut branch by numerous sporodochia on the underlying cortex. *B*, *C*, *D*, Comparison of the sooty spore deposits of the pathogen on walnut, chestnut, and lemon branches, respectively.

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MASS CULTURE OF MACROCENTRUS ANCYLIVORUS AND ITS HOST, THE POTATO TUBER MOTH[1, 2]

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INTRODUCTION

AN INSECT larva collected in Orange County, California, September 30, 1942, in the course of routine inspection work by agricultural officials, proved to be that of the oriental fruit moth, *Grapholitha molesta* (Busck). The discovery that this destructive insect had become established in California immediately created a situation which required the concerted action of entomologists and state agricultural officials. On the basis of earlier studies concerning the behavior and economic importance of this insect in other regions, the assumption seemed reasonable "that it would be a serious pest of deciduous fruit in California, particularly in view of the extensive production here of late canning peaches" (Smith, Essig, *et al.*, 1933).[6] The California State Legislature within a few months appropriated for use of the California Department of Agriculture over $800,000 for the eradication of the moth if surveys indicated eradication to be practical, and for research involving biological and chemical control (Mackie, 1944).

The surveys showed, however, that the moth was so widespread in the peach-growing areas that the proposed eradication campaign was inadvisable.

The program then resolved itself into retarding the spread of the moth into uninfested districts, the development of methods of control for use when control became necessary, and the introduction and release of insect enemies.

A part of the policy of trying to delay spread of the pest was the program of mass production and colonization of the parasite *Macrocentrus ancylivorus* Roh. in those areas revealed by the survey to be infested, since *Macrocentrus* had proved to be the most effective control of the oriental fruit moth in eastern states. The parasite appears to be an efficient searcher and it was felt to be sound policy to keep a population of *Macrocentrus* present at all times in the

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[6] See "Literature Cited" at the end of this paper for full data on citations, which are referred to in the text by author and date.

infested orchards with the idea that they would discover and destroy a large portion of the twig-infesting larvae of the pest. *Macrocentrus* apparently disperses readily. It has been shown that the adult parasites are able to travel a distance of 6 miles (Driggers, 1941; Steenburgh, 1930).

The introduction into California of the various parasites of the moth known to occur in foreign countries was also considered an important part of the biological control program. This phase of the work, however, depends on the resumption of normal traveling conditions to the oriental-fruit-moth habitats in Japan and China and on the degree to which the moth becomes a pest in California.

PREVIOUS METHODS OF UTILIZING MACROCENTRUS AND RESULTS

The oriental fruit moth was first discovered in the United States in 1915 near Washington, D.C., probably having been introduced about 1913. Within a few years it had become very destructive to peaches, particularly the fruit. Attempts to control it by insecticides proved unsuccessful. As early as 1917, however, a native parasite was observed to be playing an important part in the natural control of the moth (Wood and Selkregg, 1918). Prior to 1929 this parasite, described by Rohwer (1923) as *Macrocentrus ancylivorus,* was known to be parasitic on the oriental fruit moth only in a narrow coastal strip from southern Connecticut to southern Virginia (Allen, 1932). Here it had clearly demonstrated its value by destroying as high as 90 per cent of the twig-infesting larvae of the fruit moth. In New Jersey this parasite effected a decided reduction in the larvae infesting the fruit of the midseason varieties of peach (Allen, 1932). In Delaware during the ten-year period from 1931 to 1940, inclusive, the yearly average parasitism of the fruit moth ranged from 45 per cent in 1935 to 76.6 per cent in 1940. *Macrocentrus ancylivorus* made up 92.7 per cent of all the parasitic species noted. It is credited with the prevention of fruit losses in excess of 10 per cent (Stearns and Amos, 1941).

As the oriental fruit moth spread westward from the Atlantic seaboard, the United States Bureau of Entomology and Plant Quarantine in coöperation with various state agencies followed the dispersal of the fruit moth with liberations of *Macrocentrus.* The Bureau's Moorestown laboratory, situated in the area where *Macrocentrus* was most readily collected, served remarkably well as the source of material.

In 1928 and 1929 experiments by Driggers (1930) demonstrated that parasitism by *Macrocentrus* could be increased by the liberation of relatively few adult parasites. In 1930 mass propagation of *Macrocentrus* was undertaken in Connecticut (Garman and Brigham, 1933) using the oriental fruit moth as host. The host and parasite were propagated on sliced green apples, which permitted propagation during the winter. The apples were thinnings picked during the summer and held in cold storage. In 1932 and 1933, 12,000 *Macrocentrus* were liberated in Connecticut peach orchards.

Then in New York a method was developed which permitted the production of 26,000 *Macrocentrus* in one season. According to Daniel (1936) the procedure was to build up an infestation of the larvae of the strawberry leaf roller *Ancylis comptana fragariae* (Walsh and Riley) in large field cages and then to introduce fertilized females of *Macrocentrus*. The parasitized host larvae were collected at the time of cocoon formation and stored in rooms where the emerging parasites were easily collected. A tenfold increase of female *Macrocentrus* was obtained by this method. The number of female *Macrocentrus* that can be produced from an acre of strawberries under cage is enough for liberation over 1,200 acres of orchard at the rate of about 500 females per acre (Allen, 1942).

Brunson and Allen (1944) concluded that in New Jersey periodic colonizations of *Macrocentrus* on second-brood larvae of the oriental fruit moth increase the parasitism of such larvae and consequently effect a reduction in fruit injury by about 50 per cent. Obviously a greater reduction should be obtained if the parasite is released against the first brood. With the methods just outlined, however, it was difficult to obtain *Macrocentrus* for colonization earlier than the middle of June.

The experiences of eastern entomologists had shown that the oriental fruit moth and the strawberry leaf roller, hosts that are attacked under natural conditions, were not adaptable to continuous mass propagation. Garman and Brigham (1933) stated that "production of *Macrocentrus* on the same scale as *Trichogramma* is impossible at present." Economical year-round production of *Macrocentrus* waited on the utilization of some host that would be continually available, as was the case in the mass production of *Trichogramma* (Flanders, 1930).

Stearns (1930) stated that parasitism has not and probably never will provide an adequate control for the oriental fruit moth. Nevertheless it was obvious that the first step in undertaking the control of the oriental fruit moth in California by biological means was to attempt the utilization of *Macrocentrus ancylivorus*.

DEVELOPMENT OF MACROCENTRUS PRODUCTION IN CALIFORNIA

As the result of the distributional work of the Moorestown laboratory, *Macrocentrus* had become established on the Pacific Coast prior to 1938, apparently through releases on infestations of the lima bean pod borer, *Etiella zinckenella* (Treit.), in California, and the pea moth, *Laspeyresia nigricana* (Steph.), in Washington (Webster, 1936). *Macrocentrus* was recovered from *Melissopus latiferreanus* (Wlsm.) infesting the Catalina cherry in California and the filbert in Oregon (Dohanian, 1942).

The work of finding the host most suitable for the mass production of *Macrocentrus* was greatly facilitated by having a large supply of the parasite available when needed.[7]

One of the lepidopterous species most readily obtainable for experimental work and one which could be produced in large numbers under laboratory conditions was the potato tuber moth, *Gnorimoschema operculella* (Zell.). This moth has been notorious as an insectary pest ever since Smith (Branigan, 1916) first developed the potato-sprout method for mass culture of mealybugs as food for the coccinelid *Cryptolaemus montrouzieri* Muls. The moth often was unintentionally produced in large numbers when precautionary measures were not taken to prevent its introduction into the insectaries.

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[7] H. W. Allen, in charge of the Moorestown laboratory of the United States Bureau of Entomology and Plant Quarantine, generously fulfilled all requests for *Macrocentrus*.

In the fall of 1942 Smith suggested the possibility of using the tuber moth as a host for *Macrocentrus.* The experimental work started at Riverside with the planting of potatoes early in 1943. Shipments of *Macrocentrus* from Moorestown began in May, 1943. *Macrocentrus* females from the first shipment were placed in a cage containing potato sprouts infested with tuber-moth larvae obtained in March from the Los Angeles County Insectary. This cage was located in a basement room of the Entomology Building, Citrus Experiment Station, where the light intensity was low and the temperature was about 70° F. About 30 days later 3 females and 1 male emerged from the cocoons of the tuber moth.

Since the host larvae develop in potato tubers as well as in the potato tops, there were set up three glass battery jars with cloth tops containing infested tubers and *Macrocentrus.* The parasite reproduced as readily on infested tubers as on infested sprouts. It was then apparent that a food medium suitable for the efficient mass culture of the tuber moth, and consequently of *Macrocentrus,* was at hand, a medium that could be obtained and economically handled in large amounts and one that could be held in storage over long periods of time (Flanders, 1943).

On November 5, 1943, a mimeographed article entitled "A method for the mass production of *Macrocentrus ancylivorus* Roh." was sent to interested parties. The following February the method, considerably modified, was described by Finney, Flanders, and Smith (1944).

OUTLINE OF GENERAL PROCEDURE IN MACROCENTRUS PRODUCTION

The sequence of events from the deposition of the host eggs to the shipment of parasite cocoons requires between 20 and 30 days at a temperature of 82° F and relative humidity between 40 and 60 per cent. This sequence and the correlated life cycles of host and parasite are shown graphically in figure 1.

Mature egg-sized potatoes are superficially perforated with punctures about 1 cm apart and then placed in a single layer on a hardware-cloth tray. Infestation of the potatoes with newly hatched larvae of the tuber moth is obtained by covering the potatoes closely with a sheet of cloth having on its underside moth eggs that have been evenly distributed over the surface at the time of oviposition 3 days previous.

Young host larvae after entering the punctures are subjected to parasitization by placing the tray in a closed compartment with mated females of *Macrocentrus* for a period of 3 to 5 days. Then the trays are removed and placed in tiers above a shallow wooden container, which catches the full-fed host larvae as they leave the potatoes and migrate downward. Strips or ridges of finely crushed rock (plaster sand) on waxed plywood or sheet-metal plates are placed within the container to provide a place for the cocooning of the host larvae. The cocooning plates bearing the newly formed host cocoons are removed from the container daily. The parasite cocoons and host pupae 6 days later are freed of the host cocoons and then separated so that host-free parasites are packed and shipped into the field.

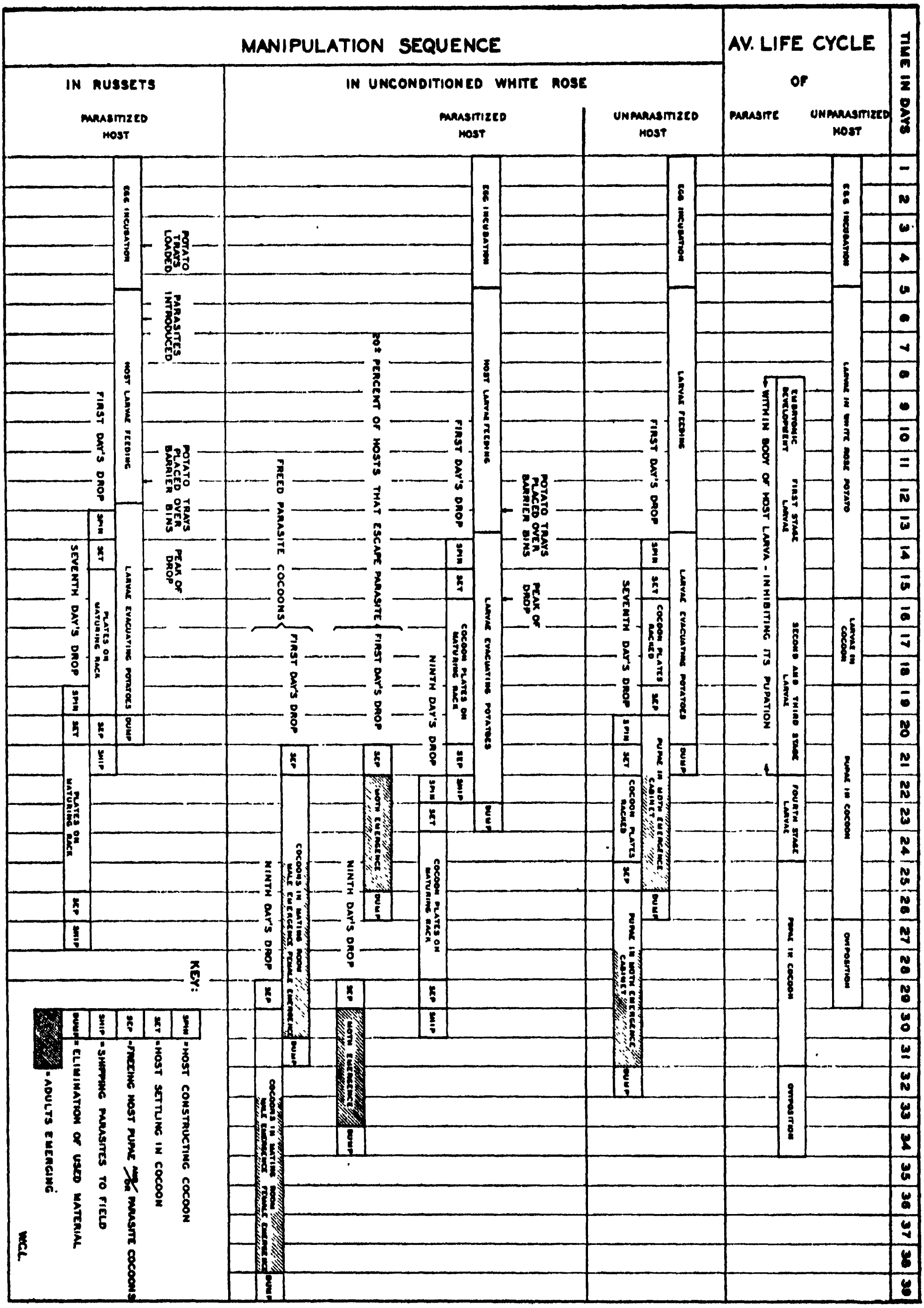

Fig. 1.—Synchronization chart of host and parasite development at a constant temperature of 82° F. Host material is equal in age but markedly unequal in development. Under certain conditions there are many slow-developing individuals, which are discarded in maintaining economy of operation.

BIOLOGY OF HOST AND PARASITE

Potato Tuber Moth. The potato tuber moth, like *Macrocentrus,* appears to be indigenous to the Americas. This is indicated by the fact that the favorite food plants, potato and tobacco, are indigenous. Furthermore, more effective insect enemies occur in America than elsewhere. The distribution of the potato around the world has been rather generally followed by the tuber moth.

The adult moth (fig. 2) is gray with a wing expanse of little over half an inch. It is most active during warm nights. During the day the adults seldom fly unless disturbed and then their flight is short and jerky. On alighting they slither into dark crevices.

The sexes occur in equal numbers. Mating takes place soon after emergence from the cocoon.

Under natural conditions the moth as a rule deposits its eggs on the leaves of the host plant so that the larva begins life as a leaf miner. As observed by Picard (1913), a roughened surface stimulates oviposition. The egg is elliptical in shape (0.45 mm x 0.30 mm). The female deposits between 80 and 200 eggs. At 80° F and 50 per cent relative humidity, most of these are laid within 3 days after emergence. At this temperature the incubation period is 5 days. During this time the egg changes from pearly white to yellow. In potato tubers the feeding of most of the larvae ceases about 10 days after hatching. The full-fed larvae leave the tuber and spin their cocoons. They pupate 4 days later. The pupal period lasts about 9 days, so that the usual life cycle at 80° F is about 28 days.

As pointed out by Graf (1917), the larval instars after the first molt are very irregular even when food and temperature are quite uniform. Larvae of the same age may be either full-grown or half-grown. Graf noted that the rate of development seemed to be affected by the quality of food rather than by the amount. Larvae in leaves and stems develop more rapidly than those in tubers. Also larvae in mealy tubers develop more rapidly than those in nonmealy tubers. This differential when in tubers, however, may be influenced by crowding (see "The Potato Tuber in Host and Parasite Production").

The variation in host development is the principal cause of variation in parasite development. *Macrocentrus* is so well adapted to its hosts—the strawberry leaf roller, the oriental fruit moth, and the potato tuber moth—that the peak of its pupation coincides with the peak of adult moth emergence, irrespective of inherent differences in the host's life cycle.

Macrocentrus ancylivorus. When adult, the parasite (fig. 3) is a wasplike insect rusty-yellow in color and about equal to a mosquito in size. The female is somewhat larger than the male and is characterized by possessing an ovipositor about as long as its body.

In the field, the adult parasites frequent vegetation likely to be infested with host larvae. When in condition to oviposit, the female explores the surface of the plant with her antennae until she contacts the webbing or frass of a host larva. She then unsheaths her ovipositor and uses it to tap the plant surface in the vicinity until the hole containing a host larva is located. The hole is explored with the tip of the ovipositor. If she contacts a larva the ovi-

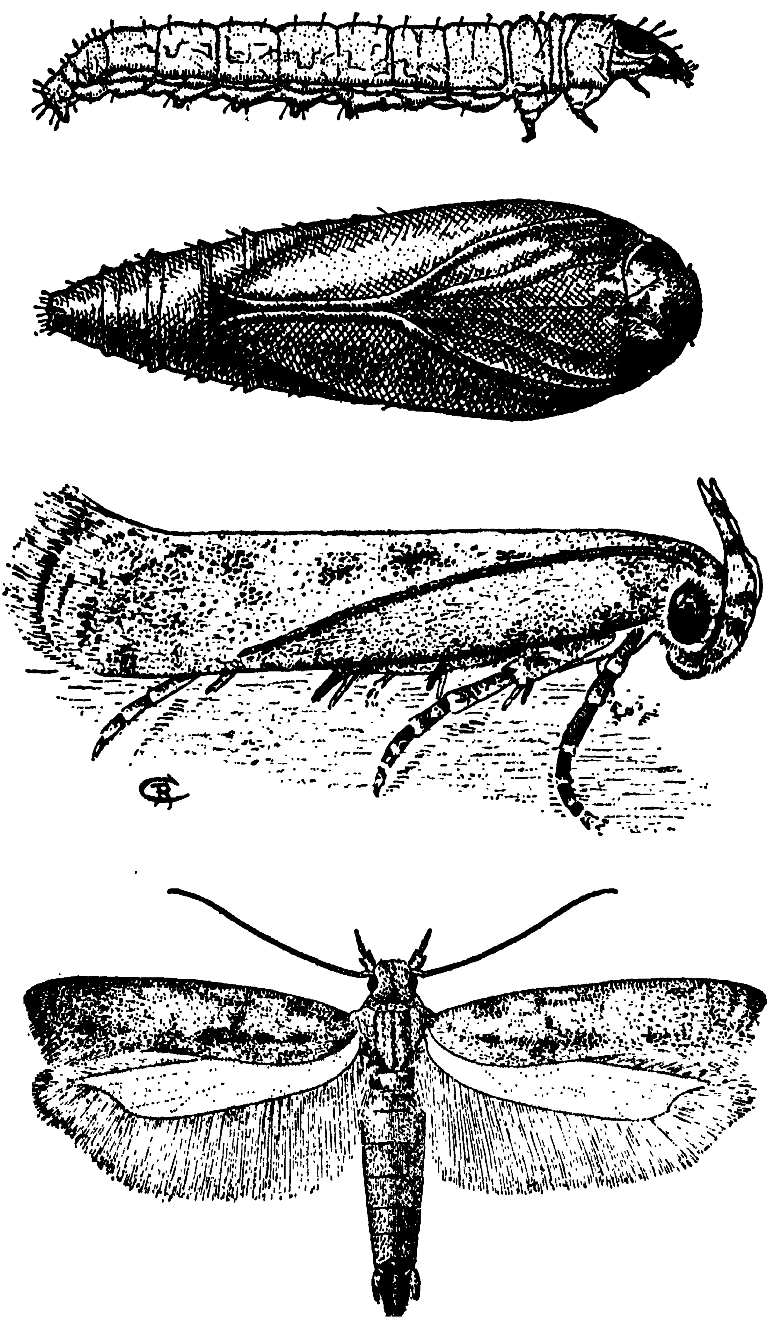

Fig. 2.—Larva, pupa, and adult of the potato tuber moth, *Gnorimoschema operculella* (Zell.). (After Graf.)

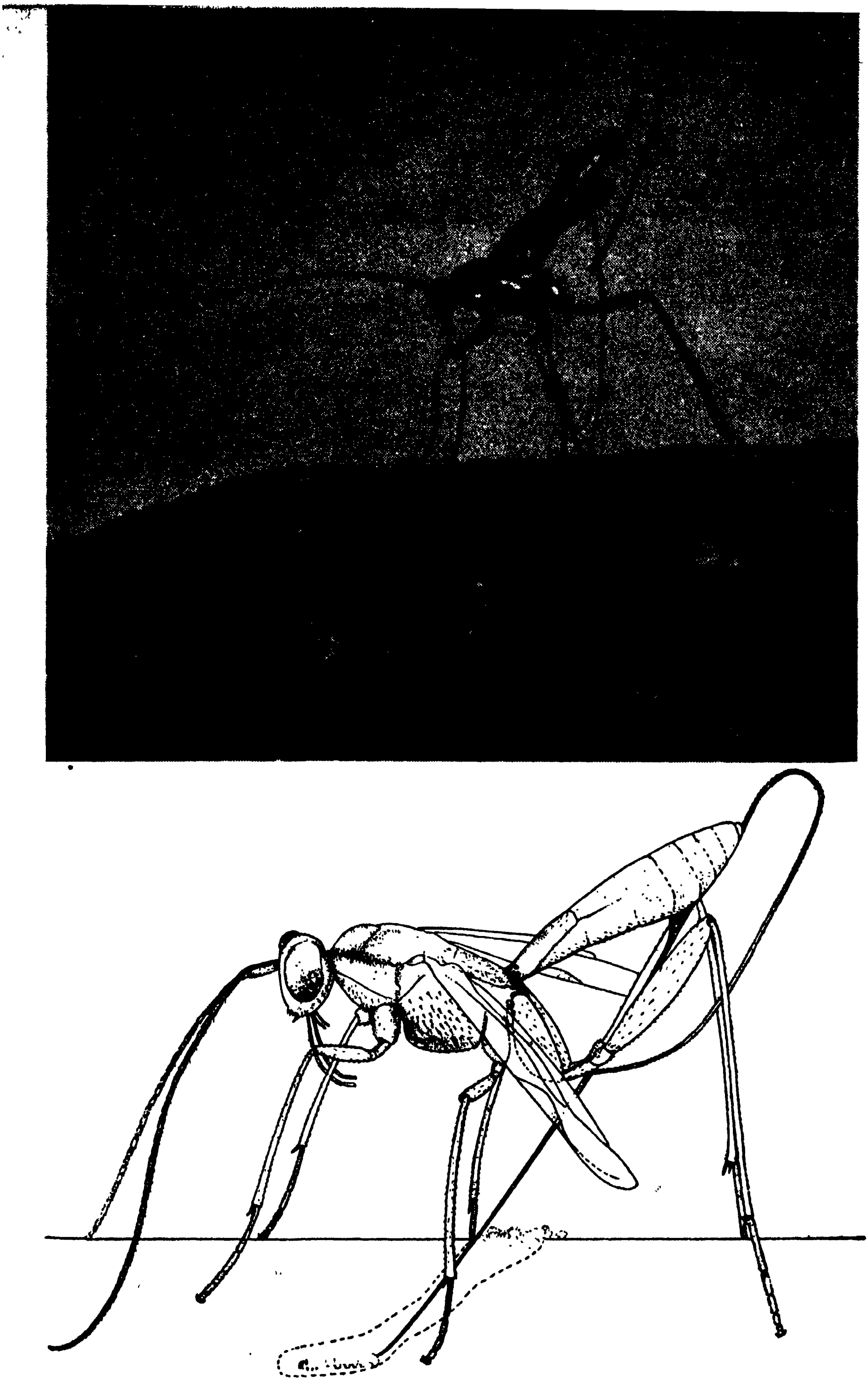

Fig 3.—*Macrocentrus* female: above, searching for the host burrow; below, in position to inject an egg into body of host. The host is attacked only while in its burrow. (*Drawing after Garman and Brigham, 1933.*)

positor quickly pierces its skin (fig. 3) and injects into its blood a minute egg, which is about 0.10 mm × 0.02 mm. As soon as the egg is immersed in blood it begins to absorb food and to grow. As Daniel (1932) has shown, however, instead of maintaining itself as a single unit, it splits polyembryonically into a number of independent parts when about 24 hours old. These consist of a preëmbryo and several pseudoembryos. Only the preëmbryo completes development, becoming a first-stage larva about 5 days after egg deposition. The number of first-stage larvae in a host depends entirely on the number of eggs deposited. But the host is superparasitized when more than 1 egg is deposited, for only one parasite can complete its development in a single host.

Macrocentrus females are capable of ovipositing about 24 hours after emerging as adults (provided the relative humidity exceeds 30 per cent and the temperature is above 60° F). One *Macrocentrus* female may be capable of depositing over 500 eggs. The maximum number of progeny of one female reared to maturity on potato-tuber-moth larvae at Riverside was 462. The female from which this record was obtained mated once and was kept at a temperature of 80° F and a relative humidity of 55 per cent. She oviposited every day for 20 days and then died of old age. The ovaries were dissected and found to be filled with eggs. Many of them, however, showed signs of disintegration. It is interesting to note that during the first 10 days the female produced 230 female and only 86 male offspring, whereas during the last 10 days she produced 146 males and no females. The first 6 days of oviposition yielded 184 females and 6 males. Such a ratio as this latter may not be desirable from the standpoint of mass culture, although 1 male has been observed to mate with 30 females during a 24-hour period. As in many Hymenoptera, unmated females produced only male offspring. Under optimum conditions *Macrocentrus* females mate only once and produce offspring having a ratio of 2 females to every male. A once-mated female produced, each day of life, progeny having the following ratio of males to females: 0 to 5; 9 to 35; 8 to 29; 6 to 19; 8 to 35; 13 to 25; 3 to 20; 6 to 11; 8 to 30; 11 to 22; 8 to 21; 7 to 11; 12 to 18; 8 to 12; 6 to 19; 0 to 4; 4 to 6; 2 to 0. The progeny developing from each day's deposition of eggs had on the average a difference of 7 days between the shortest and longest life cycles.

Macrocentrus adults emerge from their cocoons for the most part between 2 and 7 p.m. Females reach their peak of oviposition about a week after emergence; the maximum number of eggs deposited in 1 day as measured by the number of progeny was 69 (in this case 17 were males). Since *Macrocentrus* females readily superparasitize their hosts, the actual number of eggs deposited probably is considerably in excess of progeny reaching maturity. Observations indicate that the larger the host at the time of attack the greater the percentage of eggs wasted. The progeny per female decreases as the size of the host increases.

In mass-culture work, *Macrocentrus* females are utilized in parasitization for only 3 days. They are then discarded, although they have deposited only a fraction of their eggs. For the first 3 days of her oviposition period the average female deposits about 25 eggs per day (this number is based on progeny per female when 20 females were given quantities of fresh hosts daily). Eggs deposited within a 24-hour period by a female do not develop at the same rate under constant temperature conditions. In one case a difference of 17 days occurred in the life cycle of two individuals developing at 80° F from eggs deposited on the same day. The developmental rate of the parasite is modified by the developmental conditions of the host and the amount of superparasitism.

Under mass-culture conditions the host larvae are attacked 1 to 5 days after they hatch. In such hosts the life cycle of the parasite averages about 23 days, that of the male being 1 day less than that of the female. When hosts of the same age group are attacked at different times and at different stages, the parasites tend to emerge as adults at the same time. Thus the development of *Macrocentrus* from eggs laid in full-fed hosts becomes synchronized with the development of *Macrocentrus* from eggs laid in very immature larvae. This is because the parasite does not develop beyond the first instar until the time arrives for the host to spin its cocoon. Development of the parasite from then on requires an average of 13 days at 82° F.

Observations of the life cycle of each of over 4,000 *Macrocentrus* at 80° F show that in hosts that were parasitized within 3 days after hatching, the minimum and maximum life cycles of the parasites in 70 broods[8] averaged 20 and 27 days respectively, the shortest life cycle being 17 and the longest 34 days.

In the broods from hosts that were parasitized 6 days or more after hatching, the minimum and maximum life cycles averaged 18 and 25 days, respectively, the shortest being 14 and the longest 33 days.

In females that were fully impregnated, a greater proportion of the eggs were fertilized when deposited in the smaller hosts than when deposited in the larger hosts. The percentage of females from small hosts was 75, whereas that from larger hosts was 60.[9] Since the parasite contacts the host only with the tip of the ovipositor, the qualities of the host are not likely to have a differential effect on the action of the spermathecal gland and consequently on the sex ratio. The rate of oviposition probably influences the fertilization of the egg. This indicates that the intervals between ovipositions are greatest when the host is small.

The *Macrocentrus* larva develops through three stages within the host larva. According to Daniel (1932) the *Macrocentrus* larva slips out of the third-stage skin and the host larva at the same time. It then turns and feeds externally on its host, finishing in about an hour. (If this host larva had not been parasitized it would have pupated 3 days earlier.) After resting for about a half hour the parasite begins to spin its cocoon within the host cocoon. The parasite cocoon is completed in about 24 hours and consists of three layers of silk forming a capsule about 5 mm x 2 mm. When first formed it is white, changing in a few hours to glistening brown. Within a few hours after constructing its cocoon the larva pupates, forming a pupa 4.7 mm x 1.6 mm. The pupal period is 7 days. The adult may live 2 days at 80° F without food or water. Mating rarely, if ever, occurs at temperatures below 55° F (Martin and Finney, 1946*b*).

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[8] A brood as used in this paper consists of all the individuals developing from the eggs deposited by a female during 1 day.

[9] Superparasitism under mass culture may obscure the proportions of eggs fertilized (see under "Superparasitism," page 473) since the most abundant sex is the one that survives.

CRITICAL FACTORS AFFECTING MASS CULTURE OF HOST AND PARASITE

Confining the Host

Confinement of the host insect so that its activities can be controlled to suit the requirements of the project is necessary in the mass culture of beneficial insects. Since the parasite spins its cocoon within that of the tuber moth, it is necessary to control the activities of the latter in order to utilize the former.

In 1944 a production unit in the form of a box enclosing a tray was used. Four hundred such units were constructed (Finney, Flanders, and Smith, 1944). Each unit encompassed the production operations of infesting the potatoes, parasitizing the tuber-moth larvae and concentrating the parasitized larvae on sand-covered cardboards. Since the units were to be stacked in such a way as to confine the insects, a tight fit was required, and this necessitated the use of lumber that had a minimum amount of warping. Redwood was at first decided upon, but in the experimental units of redwood the parasites died without reproducing. Experiments proved that redwood was poisonous to the parasites. White pine and sheet iron were used instead. White-pine boxes and metal trays are to be preferred. Galvanized metal trays need not be painted.

In 1945 the production units were used only for infesting and parasitizing, the trays of infested potatoes being removed and stacked in the open rooms where the concentrations of larvae in the sand could be removed every 24 hours. This was possible as a result of the development of a barrier for confining the host larvae. This change cut down the number of boxes needed by two thirds and practically eliminated the problem of excess moisture (Finney, Martin, and Flanders, 1945).

Regulation of Temperature

Since insects are cold-blooded animals, their activities are to a large extent regulated by the temperature of their environment. For maximum reproduction under insectary conditions, temperatures should be used that give the highest rate of reproduction—that is, the quickest turnover of material. Once the temperatures have been established for each operation, they must be held constant so that a daily routine can be followed which will permit the most efficient use of labor and equipment. A temperature of 82° F has proved suitable for the mass culture of *Macrocentrus* and its host, although the potato tuber moth seems to attain its maximum size at lower temperatures. In order to avoid the cost of cooling, the insectary should be situated in a region where the daily outdoor temperatures rarely if ever exceed 82° F for more than an hour or two.

Use of Sand by Host Larvae

The larvae of the potato tuber moth are extremely active when they become full-fed and ready to spin their cocoons. They have a strong tendency to wander and to spin these cocoons out of reach. It was observed, however, that if these wandering larvae happen to encounter loose soil or silt they will immediately spin their cocoons, attaching them to whatever serves to support the silt provided the layer of silt is not over 3/16 inch thick. This habit, often observed in insectaries where potato sprouts were cultured, suggested that if the units containing the tuber-moth larvae were provided with cardboard floors covered with silt, the cocoons would be attached to the cardboard and would be under constant control and convenient to handle. This proved to be one of the most important discoveries in the evolution of this method of moth culture; the whole operation centers on this reaction of the tuber-moth larvae to silt, sand, or other similar material.

Various types of cocooning media, organic as well as inorganic, have been tested. Organic materials such as ground walnut shells have the advantage of light weight but react with the hypochlorite solution used in dissolving the cocoons of the host, producing heat and releasing chlorine. If ordinary soil is used, the cocoon material becomes muddy in the bath. It also contains objectionable organic material.

Sand composed of uniform-sized, smooth and spherical particles is not satisfactory, since when incorporated in the host cocoon it is not as quickly released upon immersion in the hypochlorite solution as is sand derived from crushed rock.

The Potato Tuber in Host and Parasite Production

The potato, if adequately infested, is completely utilized within 20 days at 82° F and 60 per cent relative humidity. This allows a rapid turnover of production material and thus conserves time and space and forestalls losses due to diseases and mites.

The efficient use of the tuber moth as a host of *Macrocentrus* requires the attainment of (1) the maximum number of full-fed larvae per pound of potatoes, and (2) the maximum uniformity in rates of development of larvae of equal age.

Given potatoes equal in surface area and number of punctures and infested with equal numbers of newly hatched larvae, the numbers of full-fed larvae produced vary with the variety of potato, the region or type of soil in which it was grown, the degree of maturity of the potato, and the evaporative power of the surrounding air. The most satisfactory grade of potato is small U.S. No. 1 harvested after the vines mature in areas where bacterial rot is least prevalent.

At a constant temperature and at relative humidities ranging between 40 and 70 per cent, the rate of larval development varies with the variety and size of potato, and the number of the larvae per potato. The rate is retarded if, because of too few punctures, there is excessive competition for food, or if the population is too low for optimum development. In a large potato as well as in a very lightly infested potato, larval development is so relatively slow that many larvae are lost when the potatoes are discarded. This is especially true when newly harvested White Rose potatoes are used.

At a temperature of 82° F the Russet potato will yield a much greater number of full-fed larvae than a White Rose potato of equal size and age. The larvae in the Russet mature a day earlier than in the White Rose. These varieties belong to different types of potatoes which apparently correspond to two culinary types, a mealy type such as the Russet and Green Mountain and a nonmealy type such as the White Rose and Earlaine.[10]

When newly harvested or cellar-stored Russet and White Rose potatoes are used, there is a marked difference in the pith evident 2 weeks after being lightly infested. Then the pith of the Russet is crisp and easily broken whereas that of the White Rose is leathery and tough. The most important difference, however, is in the behavior of the larval population. In the Russet the population permeates the pith, completely utilizing the tuber; whereas in turgid White Rose it remains for the most part in the cortex just under the potato skin. Consequently the host population that the latter can support is relatively small. This difference, however, is much less marked when White Rose is held for 2 or 3 months in artificial cold storage, where it is subjected to dehydration. Unless they are well conditioned, White Rose produce larvae inferior in health and vitality. Consequently they are more easily affected by adverse environmental conditions and by disease than are larvae from Russet potatoes.

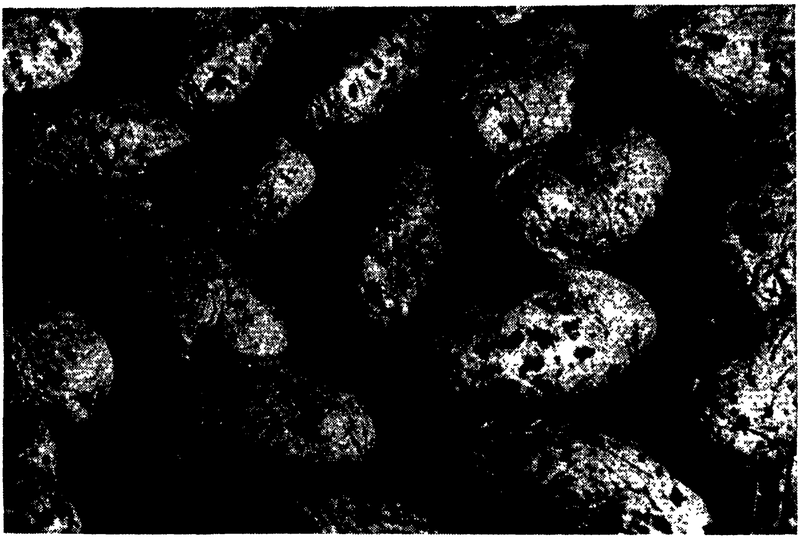

Fig. 4.—Frass or excreta of developing tuber-moth larvae pushed out onto surface of potatoes through punctures.

More frass appears to be thrown out onto the surface of the potato by the larvae in White Rose (fig. 4) than by those in Russets. This may be an effect of excess moisture in the larval tunnels. It has been observed that larvae infesting Burbank (semimealy) potatoes do not throw out their frass if the air is dry but do so if the air is humid. It is possible, however, that dry air causes the frass near the surface of the potato to harden and thus prevents its ejection. Parasitized larvae throw out less frass than unparasitized larvae. In White Rose the host larvae tend to remain near the surface or come to the surface to throw out frass where they are exposed to attack (fig. 3) to a greater extent than in the Russet.

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[10] A good mealy potato will consist of about 25 per cent dry matter and 75 per cent water. As dry matter decreases in potatoes, sogginess will increase. The range in specific gravity may be from a mean of 1.100 to 1.060 (Akeley and Stevenson, 1944). Experience has shown, however, that in the ordinary run of potatoes there is such a wide range of specific gravity that flotation is useless as a means of separation.

Macrocentrus females oviposit most readily at high humidities. Probably humidities are higher in the compartments containing infested White Rose than in those containing Russets, other things being equal. As a rule the percentage of parasitism in Russets is less than in White Rose although the actual amount of parasitism may be the same.

The White Rose potato is produced in great quantities and is available over a long period of time. If properly infested and conditioned, it yields the highest number of *Macrocentrus* per pound of potato (200± parasites per pound) with 75 to 80 per cent parasitism of host. It improves with storage; artificial cold storage reduces weight but greatly increases the capacity for producing the host. In old White Rose, wrinkled and softened by dehydration as a result of cold storage, the relative amount of parasitism is decreased, equaling the newly dug Russets from the standpoint of infestation and parasitism (170± parasites per pound; 65 to 75 per cent parasitism of host).

The suitability of White Rose, therefore, as a medium for the development of the tuber moth, measured by the increase in survival, improves with the age of the tuber (improvement is slow if held under damp conditions). This change in suitability is accompanied by a decrease in percentage of parasitism, since the actual amount of parasitism per unit is not increased. In the case of the Russet potato the suitability of the tuber for tuber-moth development is not appreciably increased by aging. As noted previously, the dehydration of Russets brings about a decrease in parasitism, both in amount and in percentage.

In order to obtain the maximum number of larvae per pound of potato, small-sized tubers must be used since they have the greatest surface area for use in infesting. Egg-sized potatoes have proved most suitable for insectary use. For efficiency in production the potato must be utilized within the shortest possible time. The feeding rate of larvae in small potatoes is greater than in large potatoes since the former lose moisture more rapidly and thus become more favorable for larval development. A high initial infestation seems to increase rate of moisture loss (Finney, Martin, and Flanders, 1945). In order to obtain such an infestation the entire skin surface of the potato must be perforated with properly spaced punctures which provide suitable points of entry for the newly hatched larvae, the number of punctures equaling at least one third of the maximum number of mature larvae that the potato can support. The dehydrating action of a heavy infestation promotes feeding toward the center of the tuber.

The diameter, shape, and depth of the punctures apparently influence the amount of infestation, but of these only depth influences the amount of parasitism. The ideal type of puncture seems to be a hole formed at right angles to the surface of the potato, the hole being 1.5 mm in diameter at the surface and 3 mm in depth. Such punctures do not tend to become closed as do slit punctures and do not become impenetrable because of the formation of heavy scar tissue.

The exposed raw tissue of the potato hardens within a few hours to form a thick scar tissue (periderm), tougher than the potato skin, so that infesting should follow puncturing as soon as possible. The manner in which the tuber-moth larvae penetrate the plant tissue and the accessibility of the larvae to attack by *Macrocentrus* appears to be determined in part by the extent of the scar tissue within the puncture. Potatoes which have had the surface scarified or in which the punctures are slitlike yield fewer *Macrocentrus* than those in which the punctures are simple round holes of small diameter such as recommended above.

The cut tissues of the Russet and old, withered White Rose potatoes seem to form scar tissue more rapidly than the newly harvested or turgid White Rose. The scar tissue on the latter, however, becomes harder and more resistant. In turgid White Rose tubers the newly hatched moth larvae appear to work into the tissue at the top of the puncture, possibly because the bottom of the puncture is too moist. In Russets, however, the larvae may penetrate the tissue at the bottom of the puncture and then work upward behind the scar-tissue walls of the punctures, where they would be more or less protected from attack by *Macrocentrus*. *Macrocentrus* does not thrust its ovipositor through plant tissues, even the most delicate. The manner in which the potato skin is cut when punctured appears to influence both the entry and the parasitization of the tuber-moth larvae. Some of the skin should be torn away.

Apparently during the early larval stages of the host it is the thickness and extent of scar tissue rather than the rapidity of the inward migration of the larvae, as suggested by Finney, Martin, and Flanders (1945), that protects them from attack by *Macrocentrus*.

The area of exposed potato flesh in the puncture may influence the number of larvae infesting the potato.

INSECTARY BUILDING REQUIREMENTS

The requirements of a building suitable for the mass culture of the potato tuber moth and its parasites are not exacting if it is located in a region where the maximum daily temperatures rarely exceed 82° F and where the relative humidities at 82° F do not exceed 60 per cent. The Albany insectary (fig. 5) is in such a location. The building should be so insulated that a constant temperature of 82° F can be maintained in the rooms at a minimum of expense.

The equipment used in production requires a relatively large amount of space for housing and handling. The ventilation equipment should be sufficiently effective to eliminate excessive heat due to solar radiation. The stratification of heated air in the production room must be prevented by adequate air circulators.

A division of the insectary into six rooms is necessary for convenience of operation. The operation and temperatures of these rooms are as follows:

1. Room for storing enough potatoes to last for at least 2 weeks; outdoor temperatures.
2. Room for the operations of puncturing potatoes, loading trays and placing them in the incubation units, and unloading and cleaning trays; temperature 70° F.

3. Room for infesting potatoes with tuber moth, parasitizing tuber moth with *Macrocentrus,* cocooning of host and parasite, housing of egg-deposition boxes, emerging of adult hosts, and incubating egg sheets. This room should be as large as the other rooms combined, without windows, with a temperature of 82° F and relative humidities of from 40 to 60 per cent according to the water content of potatoes.

4. Room for separating host and parasite and packing of parasite cocoons for shipment; temperature 75° F.

5. A small, low-ceilinged room with a window on the north side for the collecting of *Macrocentrus;* temperature 70° to 75° F. This is the only room in which a window is necessary. Philip H. Marvin in correspondence states that artificial light may be used to attract the adult parasite. A collecting screen of white cloth, about 15 inches in front of the window, is fastened to walls, ceiling, and floor.

Fig. 5.—Insectary building located at Albany, California. The production capacity of equipment installed in this building is over a quarter of a million parasites daily.

6. Room for office work, files, and so forth; temperature about 72° F.

7. A shed for housing tray sterilizer, washing rack, and trays not in use is also necessary.

PROCEDURES AND EQUIPMENT IN PRODUCING HOST AND PARASITE

Selection and Handling of the Potato Tuber

Handling Conditioned and Unconditioned Potatoes. The potatoes used for the production of the tuber moth and *Macrocentrus* should be egg-sized U.S. No. 1 grade. Potatoes grown in light sandy loam are preferable since usually they are not washed and consequently hold up well.

A mealy-type potato such as the Russet is recommended for general insectary use. Since potatoes suitable for the efficient propagation of tuber moth and *Macrocentrus* are not available throughout the season, arrangements should be made for holding the needed amount in storage for several months; the storage temperature should be about 38° F.

Russet potatoes are harvested during the fall months in the Tehachapi and Tulelake regions and may be held there at a very low season-storage rate. In late March they should be transferred to artificial cold storage. Russet potatoes keep well and may still be suitable for use during July or even until mid-August, when a new crop is harvested. It is advisable to remove potatoes from storage at least 2 weeks before infesting them.

To obtain the best results from White Rose or other nonmealy potatoes they should be thoroughly mature when harvested. This may account for the fact that the White Rose from Bakersfield, harvested early during the hot season, are much less satisfactory than White Rose from Tulelake. Nonmealy potatoes stored under artificial refrigeration seem to improve in quality more rapidly than when stored in underground cellars or caves.

It is essential to have a continuous and adequate supply of moths for infesting purposes in the insectary. These moths may be obtained either from those escaping parasitization in the parasite units or from unparasitized units devoted exclusively to moth breeding stock. When White Rose are used, particularly potatoes that are unconditioned (newly harvested), the number of moths escaping parasitization cannot alone be depended upon for infesting purposes. Hence about 15 per cent of the trays loaded daily are used solely for the production of moth breeding stock. Parasitism is low in Russets, especially if they are old and conditioned. For this reason during the major part of the season, if Russets are used, no units for moth breeding stock need be set up; all may be used for the production of parasites. The percentage of parasitism, as well as the total number produced per tray, is less variable in Russets than in White Rose. Because of this variation, when White Rose potatoes are used, more units and trays must be used solely for moth breeding stock than may be actually necessary. Since the host larvae develop more rapidly and more uniformly in Russets, the tuber is completely utilized in 16 rather than in 19 days. The more rapid turnover permits the use of over 25 per cent more trays than with White Rose in the same floor space.

The production units are used most efficiently when Russets are infested, since the Russet supports relatively large numbers of tuber-moth larvae which mature in a relatively short time with relatively little variability and relatively low percentage of parasitism. The actual amount of parasites reared per tray per generation may not equal that when White Rose are used, but the total per tray per season will be greater.

When White Rose are used, a total of 84 units can be set up with the facilities of the Albany insectary. If the White Rose are unconditioned (freshly dug) 20 of these units would have to be used for moth breeding stock leaving 64 for parasite production. These could be expected to produce about 2,000 parasite cocoons per unit, or 128,000 per day. The parasitism would range from 80 to 90 per cent. If the White Rose were well conditioned, at least 10 units would have to be devoted to moth breeding stock, leaving 74 for parasites. These would produce at best an average of 3,000 parasite cocoons per unit, or about 222,000 per day. The parasitism would average 65 to 75 per cent.

When, instead of White Rose, Russets are used, a total of 108 units can be set up with the same amount of floor space. Newly dug Russets may produce

3,500 parasite cocoons per unit, with parasitism of 60 to 70 per cent. If well conditioned, they will produce about 3,000 per unit, or about 325,000 per day; and parasitism will be 30 to 45 per cent. In either case there should be enough unparasitized moths to provide adequate infesting stock. This expanded production with Russets of course entails the use of more potatoes, and also a greater number of moth-oviposition units, potato trays, and incubation units. This probably would be worth while if a large production early in the season is considered important.

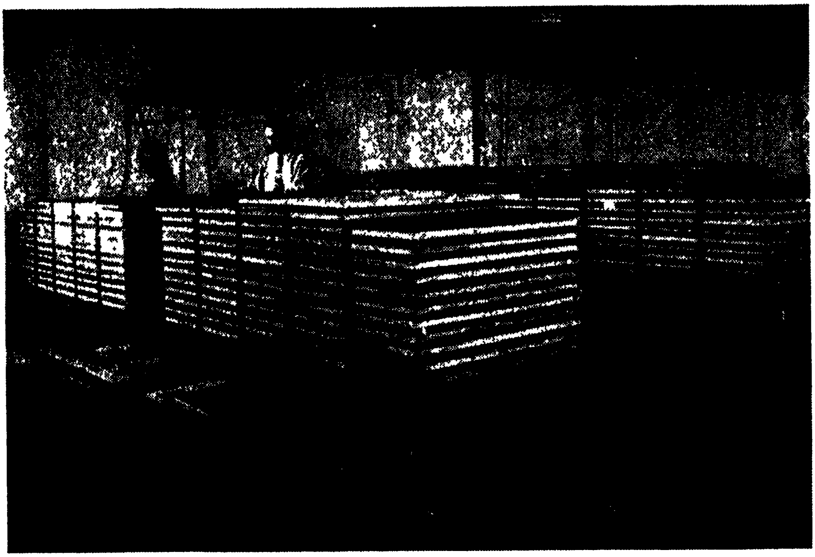

Fig. 6.—Stacks of loaded potato trays on racks set within barriers. Note cocooning plates on floor of containers. Position of hot-wire barrier in container in foreground is outlined by the white silk formed by the migrating larvae.

Bliss Triumph potatoes were given a brief trial with good results. The parasitism was between 70 and 80 per cent and the average production between 2,500 and 3,000 parasite cocoons per unit. Some extra units would be required for moth breeding stock. Triumphs did not prove to be good keepers. They might occupy a niche between the old Russets and the new crop in late summer production, since they are harvested in May in California.

Potato Puncturing Equipment. The potatoes are punctured to facilitate the entrance of the maximum number of tuber-moth larvae. The potatoes should be infested as soon as possible after the punctures are made. At present the potatoes are punctured by rolling them on a nail-studded board with a tack-studded leather mitt.

The board is a 5×8-inch piece of ¾-inch 5-ply plywood with 1-inch nails about 1.5 mm in diameter driven through it. The nails should form a pattern of 1-cm squares over an area 4 × 6 inches. In the mitt, the leather holding the tacks is 4½ × 9 inches, about ⅛ inch thick and fairly stiff. The tacks used are cut carpet tacks long enough to protrude about 5 cm out of the leather, forming a pattern of 1-cm squares over an area 3½ × 6 inches. A second piece of leather identical in size to the first is cemented over it so as to cover the heads of the tacks and anchor them firmly. The two are then stitched together around the edges. A soft leather "pocket" about one half the length of the leather pad is sewn on the top to hold the operator's fingers. A strap with a buckle is sewn across the lower end to fasten around the operator's wrist. When puncturing the potato the mitt should be manipulated with a rolling motion. The potato is placed at the end of the board farthest from the operator, then the "heel" of the mitt is placed on the potato and pulled so that the potato rolls across the nail-studded surface.

An automatic puncturing machine has been developed and is ready for use as soon as it can be modified so that it makes the proper type of puncture.

The Potato Tray. The punctured potatoes are poured onto trays (fig. 6) made of ½-inch hardware cloth fastened to a metal frame. The operator should endeavor to arrange the potatoes on the tray with as few as possible touching. This spacing, which is to prevent larvae from forming cocoons between the potatoes, is done by hand and can be very time-consuming if one is too meticulous. The potatoes remain on these trays until discarded. The trays should be so formed that there are no sharp angles or narrow crevices that will stimulate the formation of cocoons.

Infesting and Parasitizing

The Incubation Unit. Infesting of the potato and parasitizing of the larva of the tuber moth take place in closed boxes or incubator units. These units (fig. 7) are constructed so that they can be stacked snugly one on the other, the bottom of one unit then forming the top of the one below.

A unit may be made either of nonresinous wood or sheet metal. It should be large enough to contain a tray of potatoes with ½-inch clearance on all sides and at least 1-inch clearance between the layer of potatoes and the bottom of the tray above.

When in use the units need to be ventilated. This is accomplished by means of louvers in the long sides of each. Each louver is about 1½ inches wide and extends the length of the side. It is covered with gauze fastened to the box with waterproof resin glue such as Weldwood or Cascamite. The gauze retains the proper amount of moisture (60 to 70 per cent relative humidity) within the incubator unit to secure the maximum amount of oviposition. One end of each unit is provided with a hole about 1⅛ inches in diameter for the purpose of removing the egg sheets and introducing the female *Macrocentrus*. The hole is closed by a cork or cloth stopper.

The loaded potato trays are held in the incubator unit for 8 days, during which period the infesting and the parasitizing are completed.

Infesting of the potatoes with newly hatched larvae is accomplished by means of a cloth sheet (figs. 8, 9), upon one side of which the moth adults have uniformly distributed their eggs.

The trays in use in the Albany insectary hold 20 pounds of potatoes apiece. Since 20 pounds of potatoes will support an average of 7,000 to 9,000 tuber-moth larvae, the egg sheet should carry about twice that number of eggs. An excess of eggs tends to reduce the differential in infestation between the potatoes at the edge of the tray and those at the center.

A day or two before the moth eggs are due to hatch the egg sheet is placed, egg side down, on a tray of freshly punctured potatoes within an incubation unit, which is then placed in a stack.

The egg sheet should be large enough so that it touches all the tubers in the tray. Tubers that are not in contact will have few if any larvae in them. The sheet should be lightly pressed between the potatoes with the fingers. Larvae that drop between the potatoes to the bottom of the incubation unit either perish or eventually find their way up the sides of the tray where they enter the first potato they encounter. This may account, in part, for the heavier infestation at the edges of the tray.

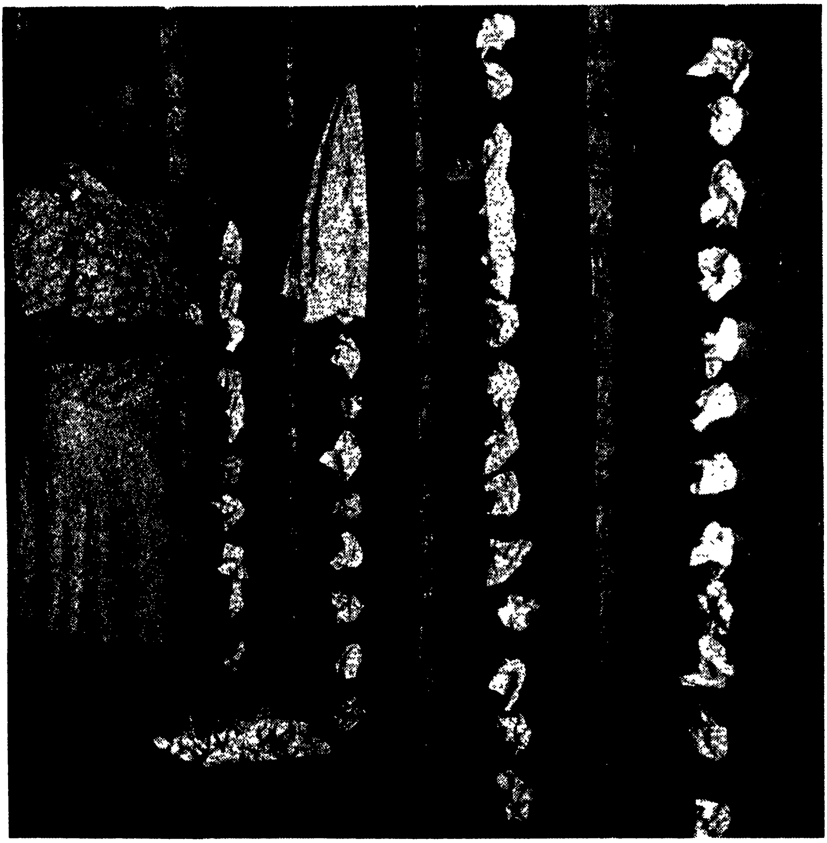

Fig. 7.—Incubation units in which infestation and parasitization occur. The apertures through which the egg sheets are removed and parasites introduced are closed with cloth-covered cotton plugs. Each platform carries two stacks of incubation units.

One or 2 days after the eggs begin to hatch the potatoes are well infested. The egg sheet in the units to be devoted to parasite production is then removed by means of a pair of long forceps through the aperture in the end of the incubation box; the honey strips are laid in, and the parasites introduced. The honey strips are prepared in advance by drawing a heavy streak of honey

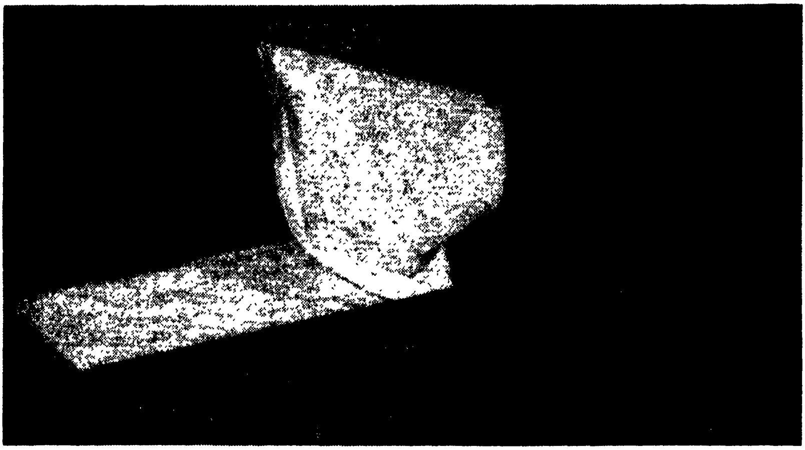

Fig. 8.—Placing egg sheet on potato tray in incubation unit. Most of the eggs hatch within one day after being placed on potatoes.

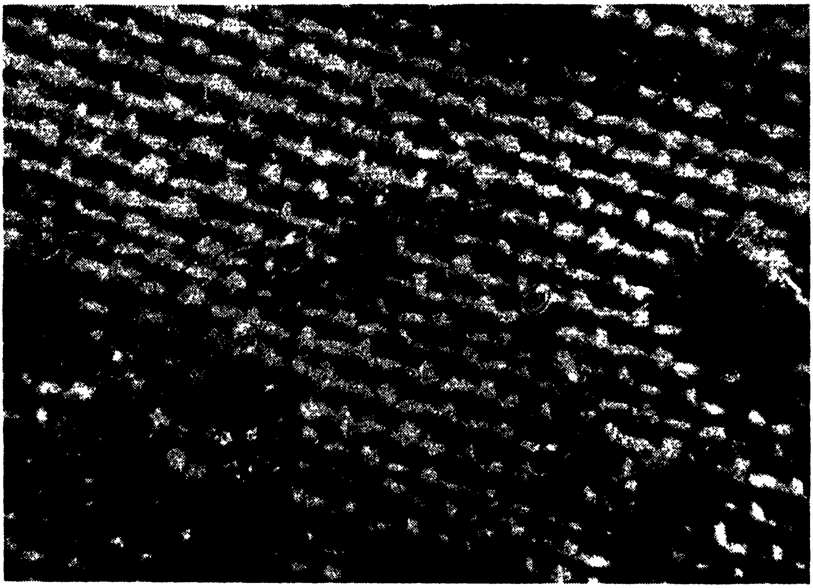

Fig. 9.—Portion of used egg sheet magnified to show cloth texture. Note tuber-moth eggs.

the length of a strip of waxed paper (1½ × 10 inches) and then covering it with another strip. When the honey strips are to be used, they are separated and then partially folded lengthwise so that they can be inserted into the units through the aperture in the end. One strip is placed on the potatoes in each unit.

With food thus provided, most of the parasites will live until the stacks are dismounted. Without food they will die within 2 or 3 days, according to the amount of moisture in the box.

The incubator unit is stocked with mated females of *Macrocentrus* at the rate of 5 females per pound of potatoes. This should be done (when Russets are used) immediately after the egg sheets are removed.

When White Rose potatoes are used the parasitization period is limited to about 3 days in order to minimize the injurious effects of excessive superparasitism. This is accomplished by delaying the introduction of parasites until 3 to 4 days before the units are to be dismantled. When the trays are moved to the racks, the parasites are allowed to disperse through the room.

Migration and Cocooning of Full-fed Host Larvae

One day before the larvae begin to leave the tubers, usually the ninth day after the egg sheets are placed on the potato trays, the incubation units are taken down and the trays placed on a rack set within a barrier.

Each rack should be large enough to hold all the trays loaded daily. Several stacks of trays may be placed on a rack. In order to facilitate the migration of the larvae and to provide suitable ventilation, the stacks of trays on each rack should be separated by about 2 inches. The trays on each stack should also be separated to allow the larvae to crawl out between them. At the Albany insectary the ends of the trays are supported by smooth sticks 1 inch square and long enough to extend beyond the length of the racks and to support the ends of several stacks of trays. The trays when stacked must be directly above one another so that the migrating larvae dropping from the edges of the tray fall directly to the barrier floor.

The trays remain on the rack for 8 to 10 days, according to the type of potatoes being used. With unconditioned White Rose potatoes, at least 10 days are required for the larvae to become full-fed and vacate the tubers; with Russets, 8 days. At an operational temperature of 82° F, using White Rose, 12 racks are sometimes needed—10 racks for trays holding parasitized infestations and 2 for unparasitized infestations.

Tray-Rack Construction. The rack (fig. 6) should be strong enough to carry the weight of trays and potatoes stacked upon it. The number of angles and niches which might induce cocooning by the full-fed larvae should be kept at a minimum. Iron-pipe construction suspended from ceiling would probably make an ideal rack. The ceiling support would allow free and unobstructed access to the cocooning plates below. There will be very little if any travel up the pipe since the larvae tend to migrate downward. The ceiling will need to be strong enough to support the load suspended from it. The stirruplike frame could be constructed so it might easily be taken down when not in use.

The rack if not suspended is provided with legs about 16 inches in length, supported by the floor within the confines of the barrier. The legs should come to a point where they rest on the floor, and if the barrier is of wood construction a small iron plate should be placed under the point to protect the floor. The points provide a minimum of contact on the floor within the barrier so that comparatively few larvae will find their way up the legs and reënter the potato trays.

Any angles or niches on the rack where the larvae tend to spin cocoons should be coated with paraffin so that the cocoons can easily be removed. This coating can best be applied by painting with paraffin-benzine solution (2 parts of saturated solution of paraffin in benzine and 1 part of benzine).

Construction of Container for Supporting Barrier and Enclosing Cocooning Plates. Under each rack is a container for trapping the larvae dropping from the trays above. The container should extend at least 10 inches beyond the rack on all sides to catch the larvae which flip outward from the stacks of trays when disturbed by one another. The floor should be constructed of 3-ply plywood, treated with linseed oil. The walls should be constructed either of Oregon pine or white pine, about 2 inches high and ¾ inch thick, and nailed and glued securely to the floor. The glue should be waterproof. The angle between the floor and the wall should be rounded to prevent cocooning there. Curved blocks should be set very solidly into each corner, eliminating any sharp angles. The curve should have a 45° arc with a 5-inch radius.

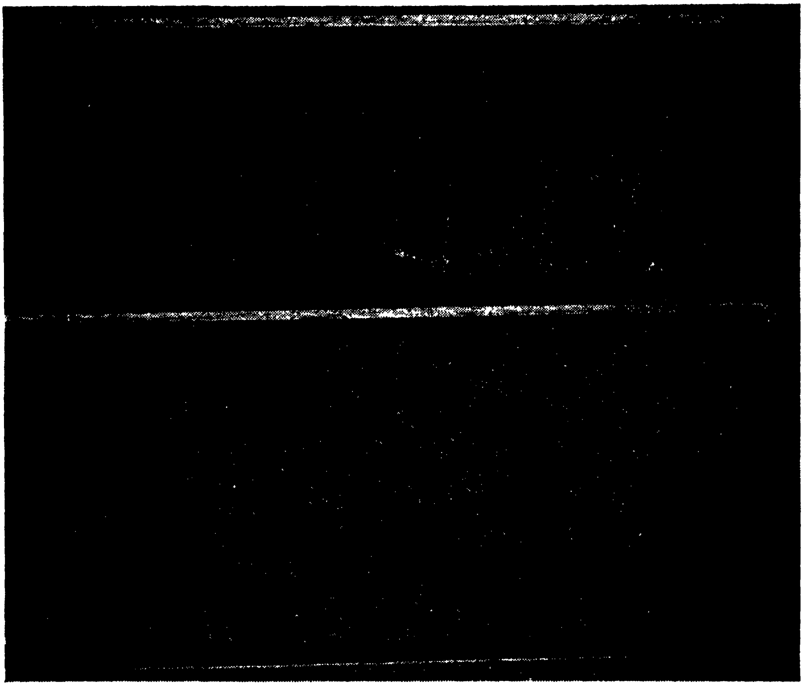

Fig. 10.—Cocooning plates showing ridge rows of tuber-moth cocoons embedded in sand.

The Hot-Wire Barrier. A barrier consisting of Nichrome resistance wire fastened by small staples is placed on the inside of the container walls. A gauge of wire should be used that will be heated to about 180° F when connected to a 110-volt line. The barriers of two containers may be connected in series if desired. A rheostat may be desirable to make possible fine adjustment of the current. The wire should not be laid on the container floor because many larvae might be injured by flipping against it. The wire should be secured about every 2 inches and run in a slightly zigzag manner. This tends to allow for contraction of the wire when it is disconnected for cleaning. The wire should be at the desired temperature while being installed. The staples should be placed so that as much as possible of their exposed surface will be in contact with the wire. A cold staple acts as a bridge for the escaping larvae.

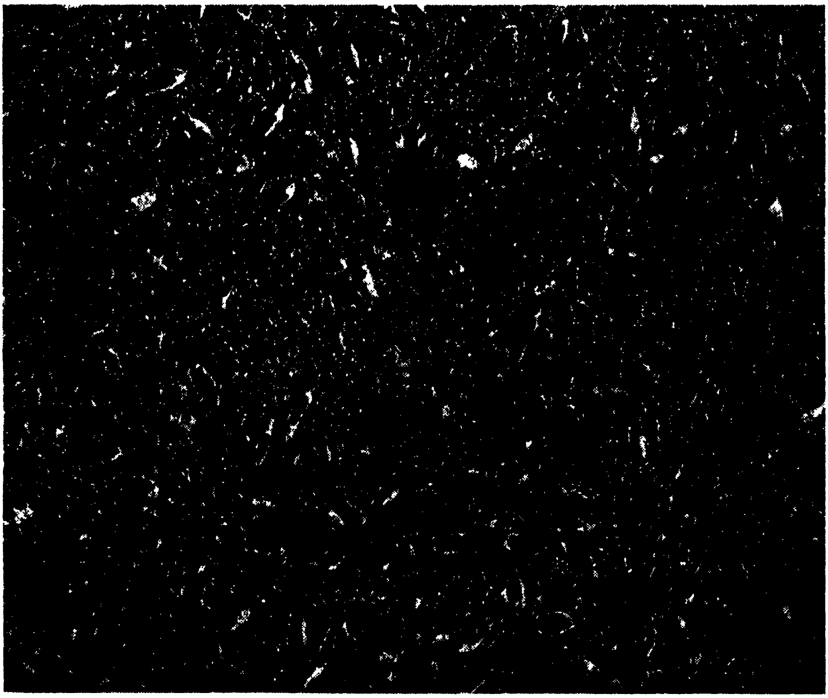

Fig. 11.—Mat of cocoons carefully scraped from cocooning plate exposing contents of many cocoons.

At the Albany insectary 23 feet of 18 B & S gauge Nichrome wire is used on each container and two are connected in series, making a total of 56 feet. When connected to a 110-volt circuit, the wire is heated close to the proper temperature.

The Cocooning Plate. The cocooning plates (fig. 10) are simply sheets of well-seasoned 3-ply plywood slightly longer and wider than the potato trays. The edges should be beveled so there is no sharp angle between the plate surface and the barrier floor. They should be made of dry wood so as not to warp. Each side should be painted with two thin coats of the paraffin-benzine solution, and aired well 2 days or more before they are used. Strips of dry sand about 2 inches wide and not more than ½ inch thick and about ½ inch apart are spread across the plate from side to side. The amount of sand on each plate and the number of plates used is dependent upon the number of larvae expected to drop on that particular day. The larvae will fill the strip solidly if it is not more than ½ inch deep (fig. 11). If more sand is needed later in the day it can be added on top of the strips already there. Judgment in the amount of sand to be used will be developed by experience.

No. 2 Monterey beach sand is used at the Albany insectary. "Plaster sand," such as is available at most rock crushers, may be used. It should be sifted through 18-mesh screen if 16-mesh screen baskets are used in the separation process. Sand can be used repeatedly if allowed to aerate and dry thoroughly.

The cocooning plates after being loaded with sand are carefully placed within the barrier. The larvae should be gently swept aside with a soft-bristled counter brush before a plate is laid on the floor.

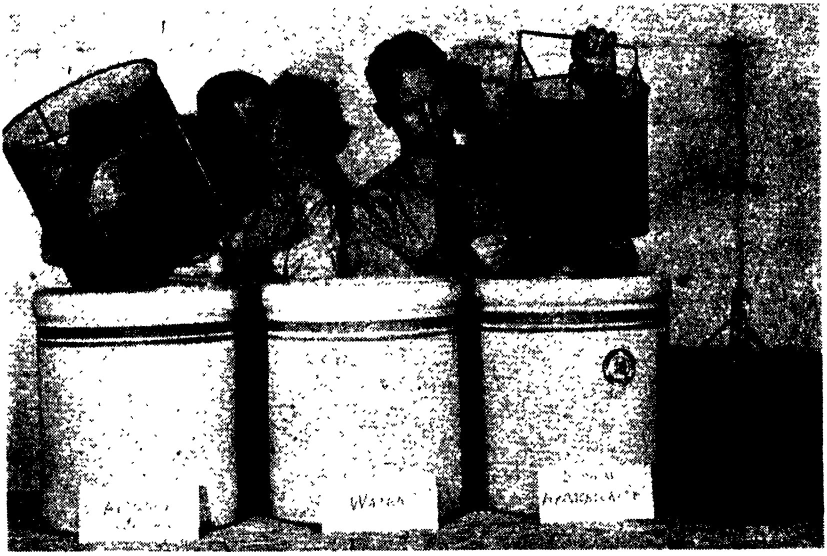

Fig. 12.—Host-parasite separation equipment. Note basket containing mass of cocoons removed from cocooning plates.

Each day the cocooning plates on which the sand is matted together with cocoons are removed from the barrier and placed over the stacked potato trays above, in order to allow any unsettled larvae to return to the newly spread sand on the plates below.

The next day, after the larvae have become firmly established in their cocoons, the plates are removed, stacked, and set aside for a 6-day period. During this period the parasite larvae leave the host larvae and spin their cocoons within the host cocoons.

Separation of Host and Parasite by Sodium Hypochlorite Process

On the morning of the seventh day after cocooning, 75 per cent of the parasites on the cocooning plates will have pupated, and the host cocoons and their contents are run through the sodium-hypochlorite-alcohol separation process in order to obtain *Macrocentrus* free from moths (Bartlett and Martin, 1945; Finney, 1945).

The silk solvent used in the separation process is made up of 1 part of a stock solution of 21 per cent sodium hypochlorite with 6 parts of water. If

21 per cent sodium hypochlorite is unavailable, a commercial bleach, containing 5.25 per cent sodium hypochlorite, bottled for household consumption, may be used—1 part bleach to 1 part water. The alcohol used is isopropyl and when diluted with water has a specific gravity of 0.92 (not Baumé). Dilution is 3 parts of 99 per cent isopropyl alcohol to 2 parts of water.

The equipment consists of three earthenware crocks (fig. 12), one for the solvent solution, one for tap water, and one for alcohol; two wire baskets (fig. 14), one of which fits loosely within the other, made of bronze welding rods and 16-mesh copper screen; a number of cloth drying frames; and an electric fan.

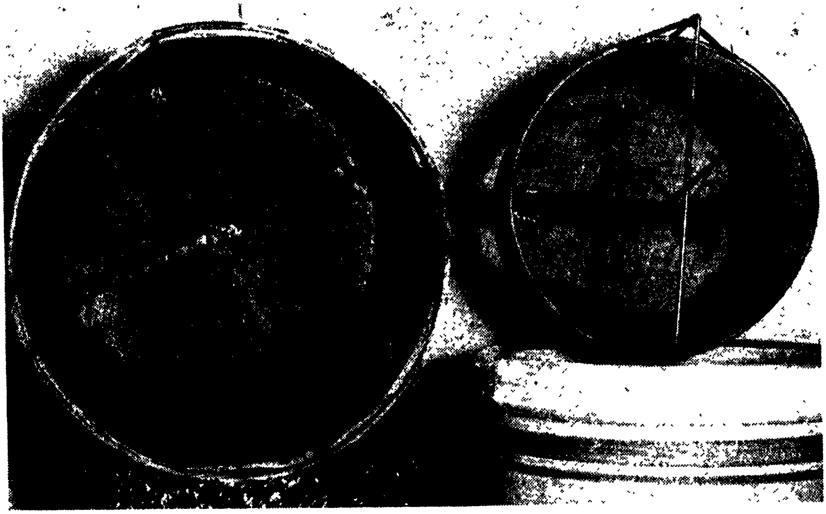

Fig. 13.—Moth pupae and parasite cocoons in bottom of wire baskets after having passed through the sodium hypochlorite solution and having been separated in alcohol.

The smaller of the wire baskets is equipped with a rigid bail over the top and with a bottom divided in half to form two flaps hinged on the middle line and opening downward. The flaps are manipulated by a jointed Y-shaped rod, the stem of which extends above the top of the basket where it is hooked over the bail.

The procedure in separating host and parasite is as follows:

The matted cocoons are lifted from the paraffined plates with a piece of sheet metal (beveled on the lifting edge) and placed in the small basket to a depth of about 3 to 4 inches. The basket is lowered into the solvent crock until the cocoons are deeply immersed. The basket is next raised slowly a few inches in the bath with a slight twisting action, then lowered quickly, so that the material is given a gentle toss and roll in the liquid. In about 45 seconds to 1 minute the host cocoons are completely dissolved and the freed sand has dropped to the bottom of the bath. The small basket, now containing only the parasite cocoons and the larvae and pupae of the host, is shifted to the vat of tap water where the material is rinsed for 10 seconds. After rinsing, the basket is lifted free of the water and allowed to drain thoroughly. Then the small basket is set within the large basket placed in the alcohol crock, the small basket being elevated so the bottom flaps will hang vertically when released. As the host pupae sink, the bottom is opened and the surface of the bath gently stirred. All the viable host pupae as well as any larvae of both host and parasite sink to the bottom of the large basket while the parasite cocoons float within the small basket.

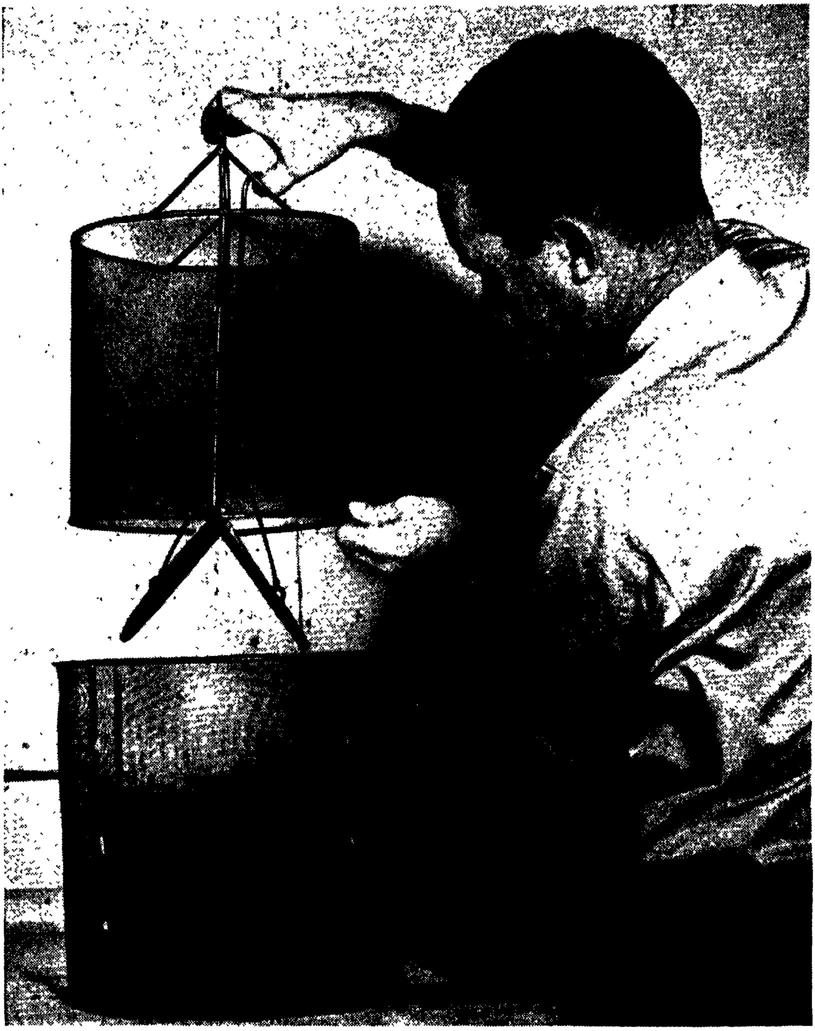

Fig. 14.—Close-up of wire baskets to show construction.

The small basket, with bottom closed, is removed from the alcohol and its contents of parasite cocoons dumped on the cloth drying frames (fig. 15). This process of separation is completed in about 20 to 30 seconds (fig. 13).

The mass of parasite cocoons is spread out thinly to dry in a current of air

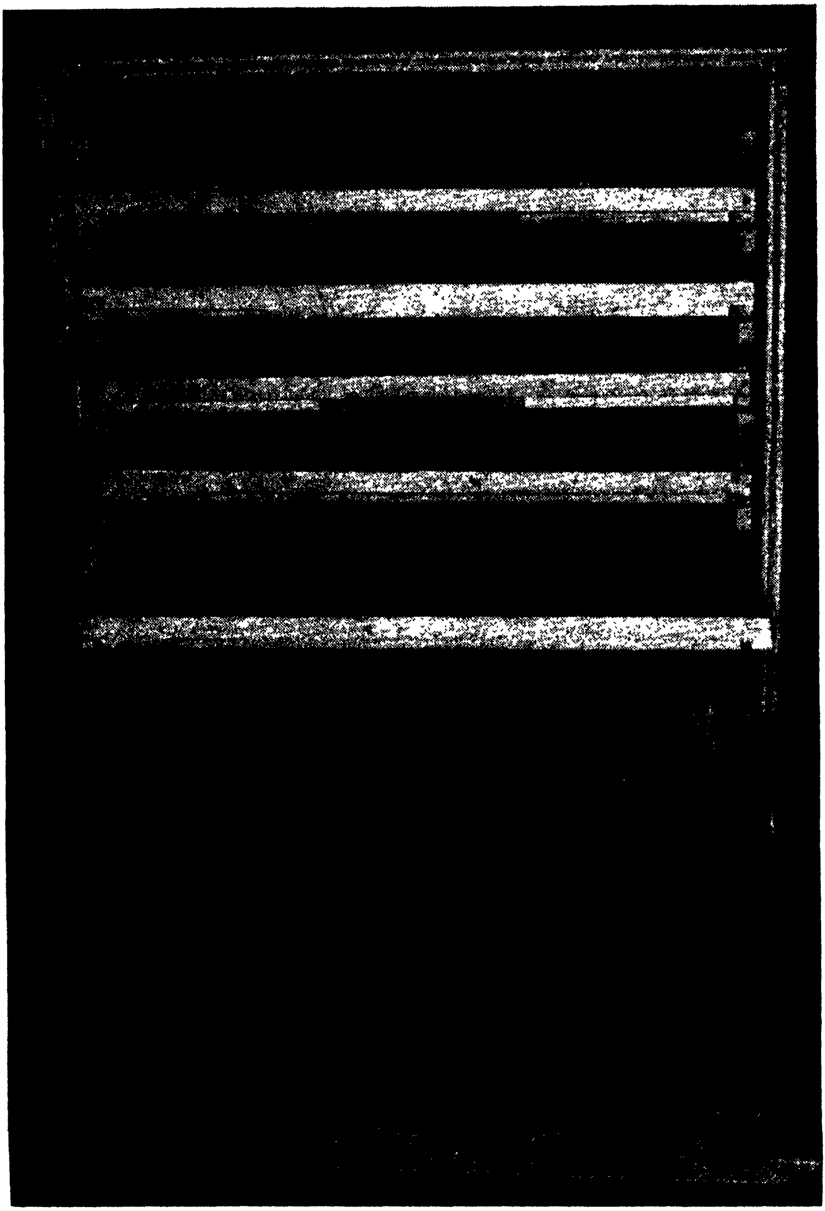

Fig. 15.—Frames holding parasite cocoons and host pupae in drying rack—one day's production.

from the fan. The large basket containing a mixture of host pupae and some larvae of host and of parasite is then removed from the alcohol, and its contents dried on cloth sheets.

The solvent solution must be used only when it is in a stable condition; that is, when there is no free chlorine being produced. Decomposition of the solution begins after a certain amount of organic material has accumulated in the vat. This organic material is derived from the silken cocoons, dead host larvae, and larval frass from the potatoes. Decomposition is indicated by the production of chlorine accompanied by a frothy scum. There also is an increase in the time required for dissolving the host cocoons and an increase in the temperature of the solution. Sufficient quantities of chlorine may be released to irritate the mucous membranes of the operators (Martin and Finney, 1946*a*).

The pupae and larvae of the tuber moth, the full-fed larvae of *Macrocentrus,* and the cocooned larvae and pupae of *Macrocentrus* can be passed through the separation process without mortality. This process has been used with equal success with several other parasites of the potato tuber moth.

Mortality of *Macrocentrus* while in the parasite cocoons averages about 18 per cent with a range from 5 to 25 per cent.

The unparasitized material from units devoted to moth breeding stock may be processed 3 days earlier than the parasitized material. Although no separation is required, drying is facilitated if the host pupae are dipped in the alcohol bath.

Handling of Host Pupae and Adults

The host pupae after separation are placed in a shallow wire basket of 16-mesh screen. If there are too many moth larvae among the moth pupae, they should be separated by flotation in a saline solution having a specific gravity of 1.05.

The baskets are set in a gastight cabinet (fig. 16), where the adult moths emerge. The cabinet should have 30-mesh screen across the top to provide ventilation but should be closed by a tight-fitting lid when anesthetizing. At the Albany insectary about 20,000 pupae (200 grams) are placed in each basket.

Anesthetizing Moths. The moths are readily handled in quantities if temporarily rendered inert—stupefied. The use of ether was suggested by Smith for this purpose. Anesthetizing of the moths is quickly accomplished by exposing them to a mixture of ether and carbon dioxide fumes. When carbon dioxide is passed, at 1½ pounds' pressure, over liquid ether in a half-filled 1,000-cc Erlenmeyer flask, the resulting mixture is nonexplosive—it will, in fact, actually extinguish a flame. A small pressure regulator with a scale calibrated from 1 to 50 pounds should be used. It should be enclosed in a metal container heated with a light bulb so that it will not become iced and cease to function properly. The dial should extend outside this box so that it may be read easily. The ether should be kept near room temperature by immersing the flask in lukewarm water. If this is not done, the flask will become iced and not enough ether fumes will be picked up by the carbon dioxide.

Two cylinders of carbon dioxide should be connected together so that a low or fluctuating pressure in one, when it is nearly exhausted, can be compensated for by the opening of a full one. This makes it possible to completely exhaust a cylinder and still retain a constant working pressure.

The air in a moth-emergence cabinet will have been replaced by carbon dioxide and ether after a 5-minute injection of this gas. A 15-second flow is sufficient for each egg-deposition box; a 20-second flow is necessary on a box-cleaning day in order to keep the moths inert the extra period of time.

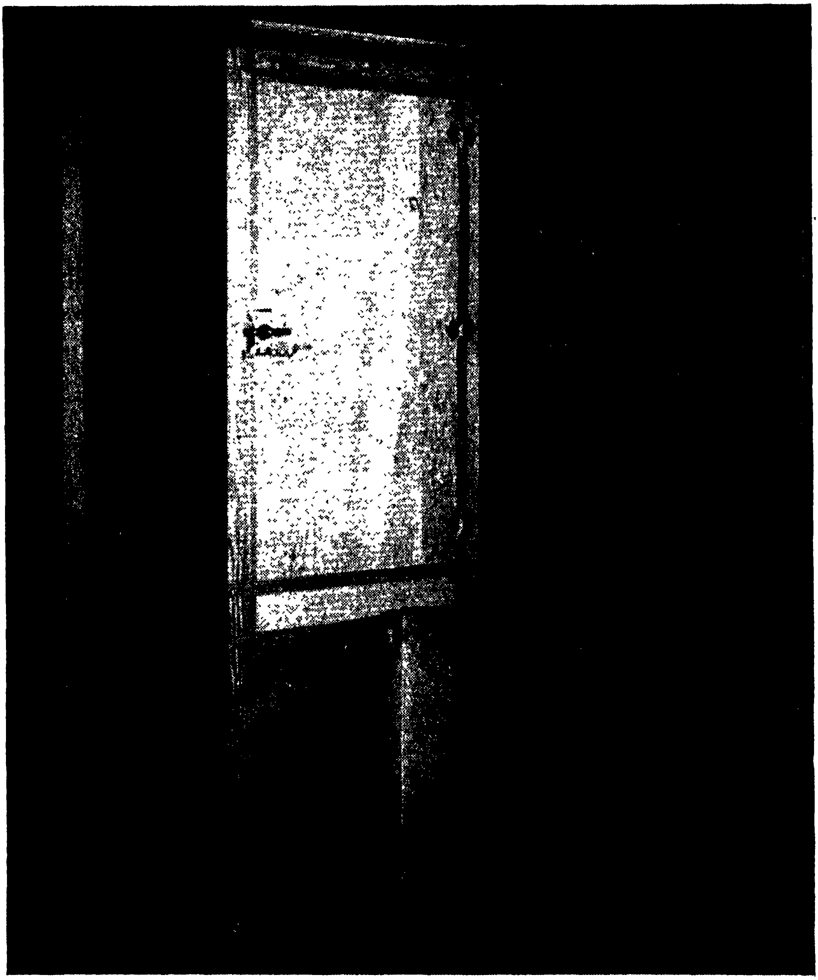

Fig. 16.—Moth-emergence cabinets, one opened to show roofed containers for holding moth pupae.

Two to 3 minutes after the air in the cabinet has been replaced by the fumes of carbon dioxide and ether, anesthetizing is complete. The drawer at the bottom is removed and the moths are poured into a trap-bottomed screen basket similar to that used in the separation process. A current of air should be directed upward through the bottom of the basket with a force sufficient to mildly agitate the moths and carry away loose moth scales and yet not spill the moths over the side. The cleaned moths can then be gently dropped through the trap door into a container having an ether-soaked sponge attached to the underside of its lid. The moths may be held inert for several minutes until ready for use.

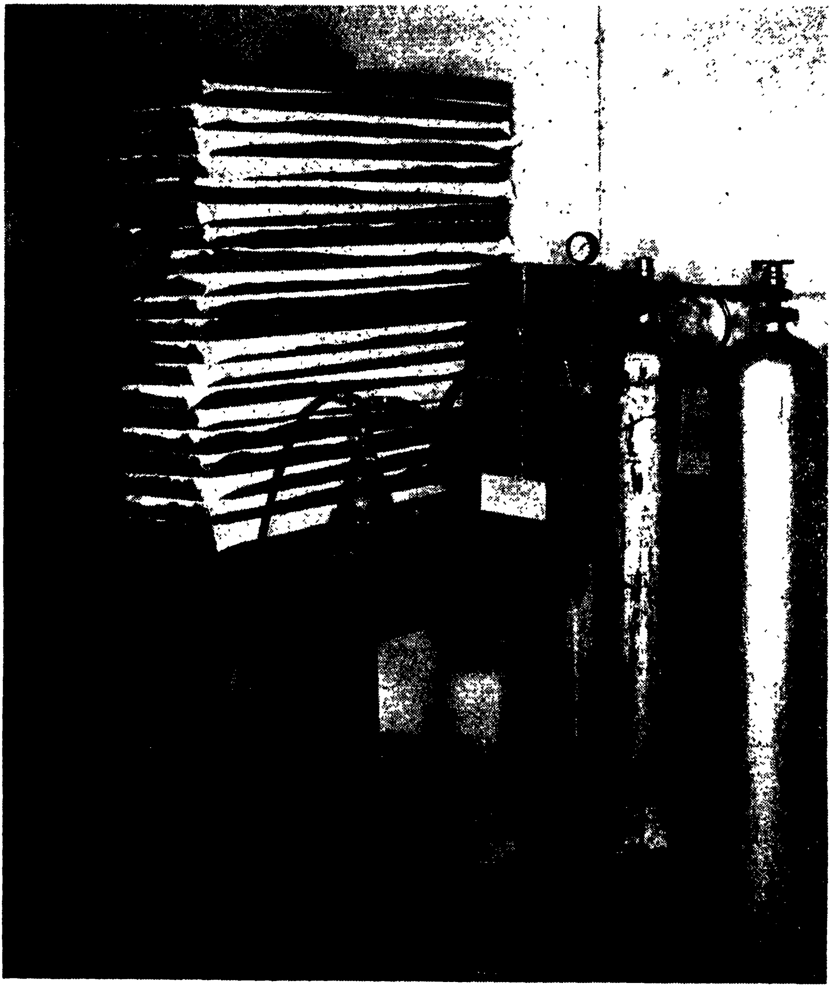

Fig. 17.—Equipment for mixing ether and carbon dioxide and injecting mixture into egg boxes.

The moths are measured into glass tubes calibrated to the amount required for each egg-deposition box. The tubes are 6 inches in length and 1 inch in diameter. One end is covered with a fine-meshed screen cap; the other is open except when the tube contains moths, at which time it is plugged with a cloth-covered cotton wad.

Construction of the Moth-Emergence Cabinet. The moth-emergence cabinet is a plywood box large enough to hold trays containing six 1-day allotments of moth pupae for insectary propagation stock. The upper four fifths of one side is made to form a door. The top is covered by a 30-mesh bronze screen and is normally open except during the anesthetizing process, when a snug-fitting plywood lid is used to retain the carbon dioxide and ether.

The box is set on legs equipped with casters so that it may be easily rolled from room to room. The legs should be long enough to raise the box to a convenient working height. A hole 1⅛ inches in diameter is drilled near the top of one side to permit the introduction of the anesthetic gas. It is at other times closed with a cork. At the bottom of the cabinet is a metal drawer into which the moths fall when anesthetized. The front of the drawer is made of wood and when closed occupies most of the remaining fifth of the space below the door. The drawer has a flange in two sides and back made to slide in grooves constructed in each side and the back of the cabinet. This prevents moths from crowding into the area around the sides and underneath the drawer.

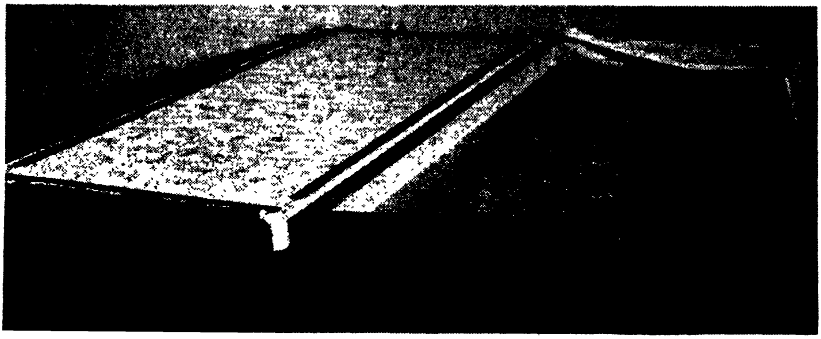

Fig. 18.—Close-up of egg boxes, to show method of fastening egg cloth. One is opened after moths have been anesthetized.

The cabinet is equipped with rods for supporting the pupa trays. Each tray is covered with a removable steep-pitched sheet-metal roof so that, during the process of anesthetizing, the moths will not fall back into the trays but will be deflected and drop on down to the drawer.

Construction of Egg-Deposition Box. An egg-deposition box (fig. 18) has white-pine sides 1½ inches high, a plywood bottom, and a removable cloth top. In one end is an opening 1 inch in diameter closed by a cork. The box should be tight enough to hold carbon dioxide. The inside of the box should be painted with white enamel paint. The top surface of the sides of the box are grooved to form narrow slots into which T-shaped metal strips are fitted, one strip to each side. The cloth top is held in place by being wedged into the slots by the metal strips. The cloth is drawn taut in the process. The cloth top consists of bleached muslin and is 3 inches wider and longer than either the egg box or potato tray. Allowance should be made for shrinkage when the cloth is laundered. It should be laundered rough dry and each sheet should be laid flat to avoid creasing and wrinkling.

Various materials have been tested for use as egg sheets, including paper toweling, blotting paper, and unbleached muslin. Suitable material must have the oviposition surface covered with a nap that stimulates oviposition, must be strong enough so that it can be drawn and held taut with a smooth, perfectly horizontal surface, and flexible enough so that it can be tucked around the potatoes in the production trays. Bleached muslin such as no. 60 longcloth has proved most suitable.

In the daily removal and replacement of the egg sheets, it is necessary to anesthetize the moths within the egg box. This is accomplished by injecting the carbon dioxide and ether mixture (which is under 1½ pounds' pressure per square inch) to displace the air in the box.

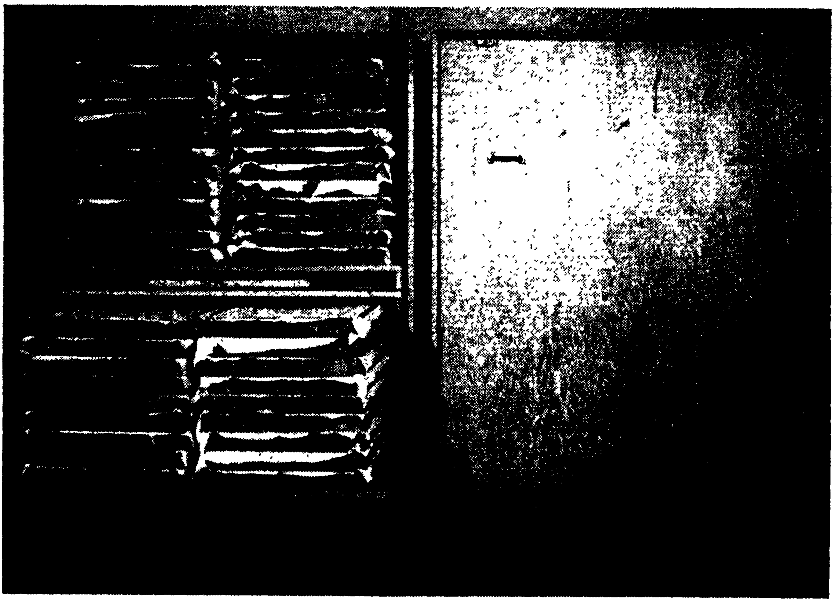

Fig. 19.—Egg-box chambers, one opened to show position of egg boxes while moths are laying eggs on egg cloth. Note two trays containing wet burlap for maintenance of 80 per cent humidity.

The steps in changing the egg sheets are as follows:

1. Boxes are removed from the egg-box humidity chamber.
2. Each box is gently upended and then turned sidewise, vertically, to concentrate dead moths in one corner.
3. Boxes are stacked one on top of the other to confine the carbon dioxide and ether mixture.
4. Carbon dioxide and ether are injected into one box after another, each box receiving gas for 15 seconds.
5. As soon as the moths in a box are "down," the egg sheet is removed and stacked (not more than 15 sheets to a stack).
6. Dead moths are removed from the corner of the box by a tank-type vacuum cleaner and the gas residue lightly fanned away.
7. A new egg sheet is stretched and clamped into position.
8. Additional moths (1.5 moths per square inch of cloth surface) are blown out of glass tubes into the boxes by air current from the compressor.

9. Egg boxes are replaced in the lightproof chamber (fig. 19), in which relative humidity is about 85 per cent.

The total number of moths in each egg box is held at about 10 moths for each square inch of the cloth top, provided the relative humidity is 80 per cent or higher.

The moth debris, which stimulates oviposition by the moths, is usually removed twice a week with a vacuum cleaner after the stupefied live moths have been poured into clean egg boxes. When this is to be done the moths are anesthetized with a 20-second application of the gas.

Handling of Macrocentrus Cocoons for Propagation Purposes

The *Macrocentrus* cocoons to be used for propagation in the insectary are placed in screen-bottom trays, like those used for moth pupae but without the roofs. The trays are placed in a lighttight emergence cabinet, the same as that used for moth emergence. They remain in the cabinet until emergence is complete (about 10 days at 84° F). From 85 to 90 per cent of the cocoons should produce parasites if they have been reared on healthy host larvae and properly handled during the separation process. With a sex ratio of 1 to 1, each 1,000 cocoons should yield 400 females. Sometimes insectary temperatures become unusually high, causing many parasites to emerge ahead of schedule. Then when the temperature lowers there is a lag in emergence. Therefore it is advisable to allot a few more cocoons than the number indicated above, so as to be assured of sufficient inoculation stock each day.

Since *Macrocentrus* cocoons have a fairly constant average size when potatoes of one variety and uniform condition are used, they can be measured by volume quite successfully. But with any change in potato type there will be a change in average cocoon size which makes it necessary to recalibrate the measuring device.

Each day the cabinet is rolled into the collecting room where the door is opened and the parasites are permitted to fly to the window of the room. The females for use in propagation are collected by suction into glass tubes identical to those previously described for holding anesthetized moths to be blown into the moth egg-deposition boxes. One hundred females (no males) are collected in each tube (fig. 20). After the necessary number of females has been collected, the remaining females and males can be removed by means of a tank-type vacuum cleaner

Before the cabinet is closed and returned to its position in the insectary, a tray containing empty cocoons is removed and one containing cocoons most recently separated is put in its place. The temperature of the room may be a compromise between a point that is comfortable to the operator and one at which the insects will be sufficiently active to leave the cabinet readily. The use of a small feather duster will assist in the evacuation of the cabinet.

At 75° to 80° F the progeny of females that emerge and mate average from 40 to 47 per cent females. The progeny of parasites emerging and mating at 84° F would be approximately 55 per cent females. Lorin Anderson, operator of the State Insectary at Palisade, Colorado, reports over 63 per cent females as a result of temperatures above 84° F during periods of warm summer weather.

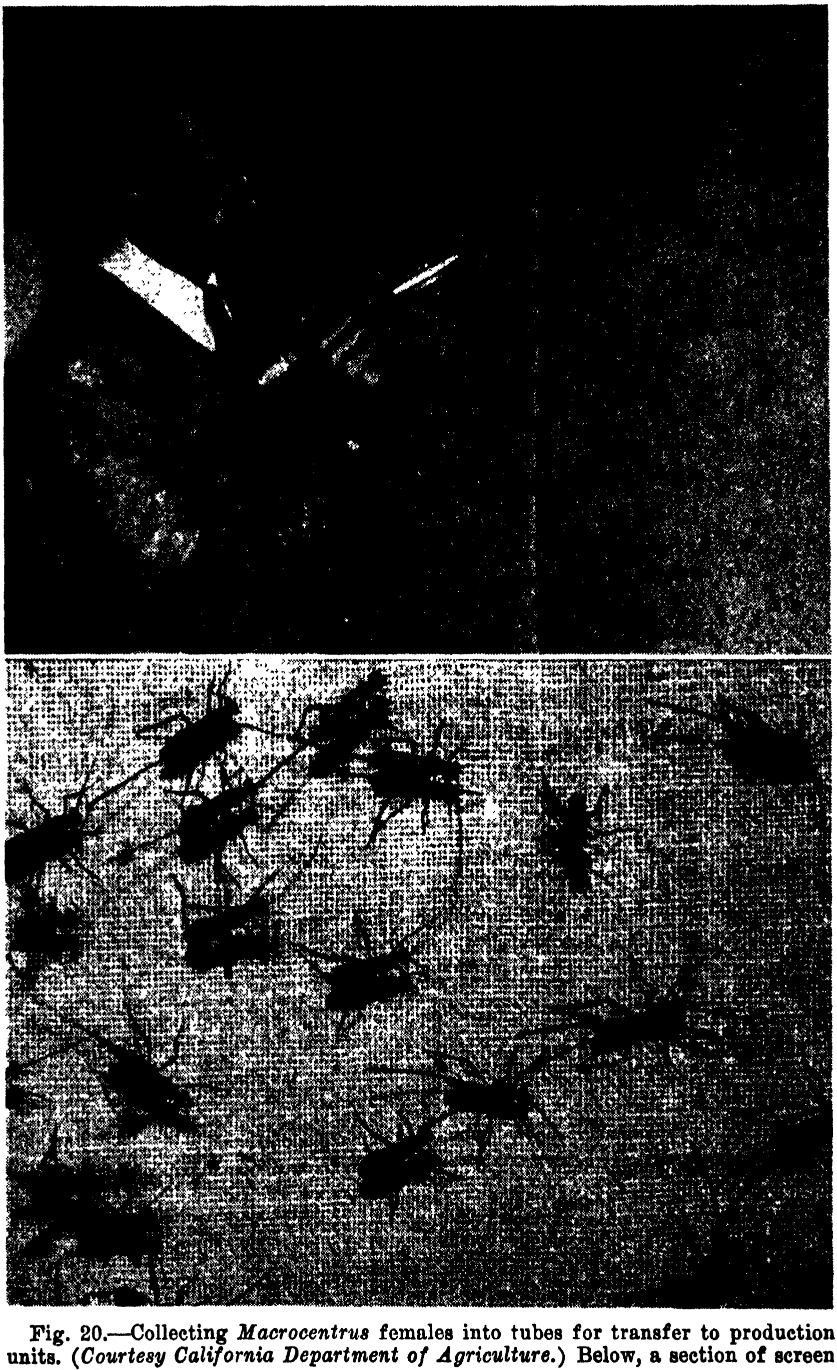

Fig. 20.—Collecting *Macrocentrus* females into tubes for transfer to production units. (*Courtesy California Department of Agriculture.*) Below, a section of screen magnified.

The proportion of eggs fertilized tends to increase with temperature when the interval between the emergence of the females and their collection is relatively short. This effect may be the result either of increased mating action or of more rapid transfer of sperm from spermatophore to sperm capsule (see the next section).

Humidity should be supplied by placing wet sphagnum moss in the drawer at the bottom of the cabinet. A 30-mesh screen on a frame capable of being easily removed for cleaning should be placed above the drawer to prevent empty cocoons or parasites from falling onto the wet moss where an unsanitary condition might develop.

FACTORS AFFECTING THE SEX RATIO

The parasite cabinet is darkened to reduce the frequency of contact between sexes. In the absence of light the parasites distribute themselves more or less uniformly. The population usually is most dense on ventral surfaces. Frequency of contact when the cabinet is dark is sufficient for the mating of the maximum number of females. With increased light intensities there is an increased amount of moving about, particularly by the males, and consequently an increased amount of mating action. Then many females mate more than once. A male may mate 30 times or more. Multiple mating tends (1) to prevent the impregnation of the females, that is, the storing of sperm in the spermatheca, and (2) to prolong the preoviposition period.

The females of *Macrocentrus ancylivorus* normally refuse to mate more than once. But under relatively high intensities of daylight, multiple mating occurs commonly, provided the density of the parasite population is such as to permit frequent contact. As Voukassovitch (1926) observed in *Macrocentrus abdominalis*, the "multinuptae" female during the first mating remains quiet, whereas in subsequent matings she moves about, dragging the male, and often tumbling and rolling. Under field conditions it is probable that a female rarely, if ever, mates more than once. An unimpregnated female produces only male offspring.

Population of once-mated females produce progeny predominantly female, the likely ratio being 1 male to 2 females. Holloway (1934) found that the usual ratio under field conditions was 2 males to 3 females. The greater proportion of males in the field may be the effect of oviposition preceding mating. When laboratory conditions are optimum for impregnation, the numbers of females produced should outnumber the males nearly 2 to 1. In one test 60 impregnated females produced 2,846 offspring having a sex ratio of 1 male to 2 females.

When a female mates, it receives from the male a relatively large spermatophore, which must be discarded before an egg can be laid. At a temperature of 80° F, 6 hours are required for the transfer of all of the sperm from the spermatophore to the sperm capsule. At 84° F this transfer probably is completed in less time. This would mean that when the collecting-room temperature is kept at 84° F a greater proportion of the parasites are ready to oviposit at the time of collection than when the operating temperature of the room is 80° F or lower.

If the spermatophore is not emptied at the time of collection it may be dislodged during the process so that the female does not receive a full supply of sperm.

Multiple-mated females may contain 2 to 10 spermatophores or more. Nevertheless, half of such females that oviposit may produce only male offspring. The preoviposition period of such females may be prolonged several days (usually less than 5 days) because of the additional time required to discard excess spermatophores. Females in which the preoviposition period exceeds the period allowed for parasitizing in the inoculation units produce no progeny.

Once-mated females may not oviposit for 12 hours after mating because of the presence of the spermatophore. As high as 20 per cent of ovipositing once-mated females may have only male progeny, either because they are unimpregnated or, if impregnated, because their spermathecae do not respond to stimuli from the host.

SUPERPARASITISM

Sometimes, when unconditioned White Rose potatoes are used, as many as 4 per cent of the *Macrocentrus* larvae are lost because they fail to cocoon before being run through the separation process. It is inadvisable to attempt to save this 4 per cent because separation too long delayed results in loss of moths due to emergence before separation and in shortening the shipping period. Superparasitism may be one of the causes of late cocooning. Few superparasitized larvae complete their development. These die and turn black and therefore are discounted. At the end of the 3-day parasitization period 50 per cent of the host larvae may contain from 2 to 28 first-stage larvae of *Macrocentrus*.[11]

Eight to 10 days are required for the parasitized host larvae to evacuate the potatoes completely. With White Rose potatoes the percentage of hosts parasitized as they leave the potatoes may vary from 39 on the first day, to 56 on the third day, 72 on the seventh day, and 93 on the tenth day. With Russet potatoes the percentage parasitized may vary from 20 on the first day to 85 on the fifth day.

The average ovipositing female deposits about 20 eggs per day at a constant temperature of 80° F so that during the 3-day period of parasitizing, when the inoculum consists of 100 females, as many as 6,000 eggs may be deposited in the larvae infesting the potatoes on a single tray.

Superparasitism by once-mated females if not excessive may be advantageous since it tends to increase the proportion of females (Martin and Finney, 1946*b*). Competition between the sexes occurs only during their first instars. Since there are more females than males in this competition the females are more likely to survive. But when the inoculum includes unimpregnated females, superparasitism may be very disadvantageous because of the excess of males eggs deposited (Flanders, 1945*a*).

Prolongation of larval development resulting from superparasitism may account for the fact that among the progeny of a female that oviposited in 24 hosts in a 24-hour period, there was a 16-day differential in life cycles at a constant temperature of 80° F.

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[11] These data and those in the following paragraph are from unpublished observations by Charles H. Martin.

PACKING, SHIPPING, AND COLONIZING

Small paper bags (size SS ½) are used for the shipment and liberation of *Macrocentrus* (fig. 21). A loop of string is attached to the top of each bag so that it can be suspended in the field.

The cocoons of the parasite are poured into the bags, about 1,000 per bag. A loose wad of excelsior is next placed in the bag on top of the cocoons. The bag is closed by folding the top. It is then fitted snugly into a shipping container.

Each day a sample of 100 cocoons is set aside for rearing in order to obtain the sex ratio and per cent emergence. The value of *Macrocentrus* releases depends, in part, on the proportion of females.

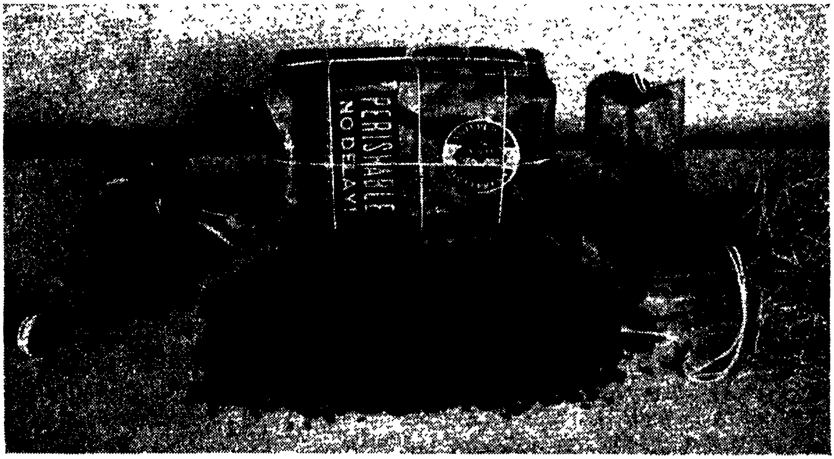

Fig. 21.—Equipment for packaging parasite cocoons for shipment.

Since *Macrocentrus* disperses readily, the bags are placed on every tenth tree. Before suspending the bag, it is opened and the wad of excelsior pulled up to keep the top open. Many of the adults crawling through the excelsior are able to rid themselves of their cocoons, to which they would otherwise have remained attached. *Macrocentrus* begins to emerge from its cocoon about 5 days after passing through the separation process if held at 82° F. Adults may continue to emerge for a week or more after the cocoons are placed in the field, the period depending on existing temperatures. They are placed in the field at the rate of 2,000 per acre per week.

In regions of frequent rainfall it may be necessary to use bags of cheesecloth. The bags should be placed where the danger of attack by hyperparasites or predators is the least.

The transportation of *Macrocentrus* from insectary to orchard, often over distances of several hundred miles, is an important phase of the work. In the beginning of the project the cocoons of the parasite were shipped while within the host cocoons. It was practically impossible not to include a few tuber moths. Although there was no real harm in this, it seemed better to avoid the shipment of pests if for no reason other than that it might be misunderstood by some orchardists.

It was found that anesthetized adults of *Macrocentrus* could be held under refrigeration for 4 days without injury. The shipment of such adults, however, required rather expensive insulated shipping units which were not readily obtainable (Smith, 1944). The problem of transportation was solved by the development of the host-parasite separation process, which permitted shipment of the parasite while still in its cocoon, free of its host, and over long distances (Bartlett and Martin, 1945).

As Garman and Brigham (1933) have shown, *Macrocentrus* does not oviposit at temperatures below 60° F and is sluggish in action at temperatures below 65°. This habit may limit the successful utilization of *Macrocentrus* to regions where the temperatures during the greater portion of the time are above 65°.

SANITATION

Sanitation measures rigidly enforced will prevent losses from such pests as the mite *Pediculoides* sp., the chalcid *Dibrachys* sp., the red disease, and the black disease (Steinhaus, 1945).

The most effective measure is the rapid turnover of material and its proper disposal afterward. All discarded potatoes, before they are carried away to a dump, should be fumigated with methyl bromide in a chamber large enough to accept one day's disposal.

The hot-wire barrier if properly placed and cared for is 100 per cent effective in preventing larvae from escaping and spinning up in crevices where they may be found by *Pediculoides* or *Dibrachys.*

The potato trays are cleaned by immersion in boiling water (fig. 22) until the cocoons, dried frass, and other debris are sufficiently loosened to be readily removed through the flushing action of water under pressure. At the Albany insectary, the trays are boiled for 15 minutes. The hot-water treatment used in cleaning the potato trays is also effective in eliminating material in which pests may breed.

Dry-cleaning the trays with a stiff-bristle brush was found to be slow and unsatisfactory.

The red and black diseases of the tuber-moth larvae cannot be completely eliminated but may be reduced to less than 1 per cent by the use of Russet potatoes or well-conditioned White Rose free from rot. More than 85 per cent of the moth pupae obtained are viable. The red and black diseases may be curbed by preventing high humidities in the cocooning room where larval feeding is at its peak. Temperatures above 87° F in the incubation or cocooning rooms will cause an outbreak of the red disease. If this temperature should persist, the loss due to this disease could be serious. High temperature seems to be the prime factor causing epidemics of the red disease.

In the late fall and winter of 1946–47 there was a lowering of the percentage of emergence of both moths and parasites and also a reduction in the percentage of parasitism. During February the host emergence dropped to nearly 50 per cent and parasite emergence was down to between 65 and 70 per cent. Material examined by E. A. Steinhaus showed that 45 per cent of larvae still within the potatoes contained microsporidian spores. Of the evacuated larvae 20 per cent contained spores, a fact which indicates that many infected larvae perished within the tuber. Protozoa were found in 20 per cent of the host pupae and 20 per cent of the parasite larvae and pupae.

Several sheets of eggs were treated by immersing the eggs for 20 minutes in water heated to 118°–119° F (Allen and Brunson, 1947). When eggs were treated, 95 per cent of host pupae emerged, as contrasted with 50 per cent from untreated eggs. Host production rose from less than 4,500 per unit to more than 10,000 per unit. From cocoons reared on hosts from heat-treated eggs, 89 per cent of parasites emerged, as compared with 64 per cent from hosts reared from untreated eggs.

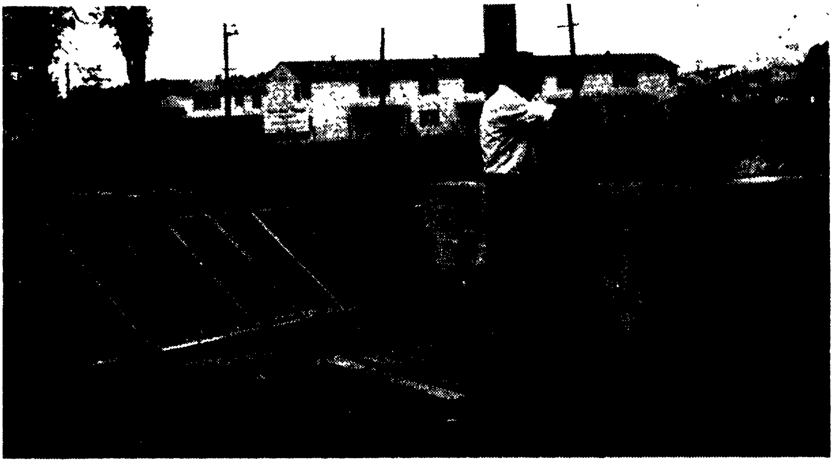

Fig. 22.—Dipping potato trays in hot-water sterilizer to destroy pests and loosen any cocoons that adhere to trays. Trays are placed on tilted racks for washing.

Allen and Brunson (1945) report a considerable number of adult *Macrocentrus* of both sexes attacked by a microsporidian. The heavily diseased individuals were characterized by conspicuous whitish areas on the abdomen. In such cases the abdomen becomes smaller and malformed. The life span of such an individual is shortened.

Allen and Brunson found the progeny of diseased and healthy adults of *Macrocentrus* to be equally affected. Infection apparently occurs in the digestive tract while *Macrocentrus* inhabits the host and adults may manifest it several days after emergence. As noted by McCoy (1947) the presence of the microsporidian in *Macrocentrus* "is not as a true disease but more by accident."

COST OF PRODUCING MACROCENTRUS ANCYLIVORUS

Season of 1944. Tables 1 and 2 give the figures for colonization of *Macrocentrus* for the year 1944, by counties and by months. As shown, the total number of *Macrocentrus* colonized during the year was a little over 19 million. This does not include the parasites used as parent breeding stock, or those used for experimental work. The actual production amounted to well over 20 million. The figures in the tables are estimates, but it is believed that the possible error is not in excess of 10 to 15 per cent. At the Albany insectary the figures were secured by estimating the number of moth cocoons per cocoon sheet, and then multiplying this number by the fraction that was parasitized. The latter figure was obtained by removing 1-inch-square sections of the cocoon sheets and actually determining the fraction of moth cocoons that contained parasite cocoons. At the Riverside insectary, the adult parasites were colonized, rather than the parasite cocoons, and the number of adult parasites was estimated at the time of collection.

Collection of the parasites, from an illuminated cloth screen, into no. 12 paper bags lightly filled with excelsior, was accomplished by the use of a large aspirator having a mouth 1½ inches across. The cloth screen was marked off in 6-inch squares, and practice made it possible to estimate with sufficient accuracy the number of parasites on each square.

The cost of producing the parasites may be considered from several different angles. Most likely to be of interest to an entomologist, however, is that cost for which, with established techniques, he might reasonably expect to be able to produce these by the thousand, omitting charges for research. The principal items entering into this cost are housing and services, equipment, salaries and wages, and supplies (primarily potatoes).

The cost of housing and of services varies so greatly, not only with the locality but with the requirements of the individual entomologist, that no figure is given here. At both Riverside and Albany, structures which have been built for other purposes were remodeled to provide the necessary conditions, such as temperature and ventilation, for use as insectaries.

The production units described in the previously mentioned article (Finney, Flanders, and Smith, 1944) were used with satisfaction throughout 1944. The cost of the complete units (box and tray) varied greatly. At Riverside, wooden units were used, and these, including painting, cost around $7.00 each. The units used at Albany were constructed entirely of galvanized iron and cost, roughly, $2.75 each. Most of the workers seemed to prefer the wooden units, but these certainly were not sufficiently superior to justify the extra cost.

At Riverside, 400 production units were operated; at Albany, 1,500. The total cost of these units, with which approximately 19 million parasites were produced, was about $7,500. There is no reason why such units should not last several years. Depreciation charges should be calculated accordingly, for addition to production costs.

Labor is an important cost item in mass production of most parasites. Considerable progress has been made, however, in reducing this item by mechanization wherever possible.

Salaries chargeable to production were estimated at $5,000. This amount would provide for an insectary superintendent and an assistant superintendent. Since the work must be done every day in the week, two men are required for supervision.

Labor costs for the production of the 19 million *Macrocentrus* in 1944 totaled, roughly, $7,335. The labor was mostly female, at the rate of 75 cents per hour.

The principal expendable supply used in the mass production of *Macro-*

centrus was potatoes. For the 19 million *Macrocentrus*, 1,890 sacks (189,000 pounds) of potatoes were used, at an average cost, exclusive of transportation, of about 1½ cents per pound, or $2,835. For parasite production for orchard colonization, potatoes were used at the rate of 15 pounds per unit, an average of 63 units being loaded per day, for a period of 200 days. The low cost of the potatoes was due to the fact that so-called peewees and egg-sized tubers were found to be most suitable, and were considerably cheaper than the regular commercial grades.

The principal items in the cost of producing 19 million (table 1) *Macrocentrus* for orchard colonization in 1944 were therefore as follows:

Salaries	$ 5,000
Wages	7,335
Potatoes	2,835
Total	$15,170

For these items, the cost per thousand parasites was about 78 cents. To this cost would also have to be added proportionate charges for certain miscellaneous items such as freight on potatoes, depreciation of building and equipment, supplies such as cardboard and sand or other material for cocoon sheets, and services such as heat, light, water, and telephone.

Season of 1945. The colonization of parasites reached a total of 15,935,000 in 1945, as compared with 19,295,000 during 1944. The drop in production over the previous year, as will be seen by a study of table 1, occurred during June, July, and August. This slump in production was the combined result of a number of unfavorable developments which, at that time, could not have been foreseen, as follows:

1. Failure of the supply of breeding moths. This was caused by an unexpectedly high percentage of parasitism in newly dug White Rose potatoes. This shortage of moths alone cut to one third the number of moth eggs available for the next cycle of production.
2. Use of one bad lot of cheap, unconditioned White Rose potatoes, which was heavily infected with rot and disease. Mortality was high; the few insects that reached maturity were weak and unhealthy.
3. A lapse of 5 days during which no trays were set up, because the Army had taken over all supplies of potatoes.
4. Failure of the potato-puncturing machine to create punctures suitable for obtaining high parasitism.

During the 1945 season all the *Macrocentrus* production work was carried out in one insectary, at Albany. Approximately 2,200 sacks of potatoes were used, at a cost of roughly $5,500. A better grade of potatoes was used in 1945 than in 1944. Labor costs were somewhat greater in 1945, due partly to higher wage scales, and amounted to $8,835. Costs per thousand *Macrocentrus* were therefore considerably higher than in 1944. But if the summer slump in production had not occurred, costs in 1945 would undoubtedly have been lower than in 1944.

Season of 1946. The total number of parasites shipped from the Albany insectary in 1946 (April to October, inclusive) was 22,935,000. The total produced was 28,812,000. This number, however, was not the maximum possible with the equipment used, since during the last part of the season the potatoes (White Rose) were not properly conditioned for maximum production. The potatoes were held in the caves at Tulelake for several months before it was realized that such storage did not properly condition them.

The value of the 1946 colonization was considerably enhanced by the fact that the number of females exceeded the number of males. This fact also means that the number of cocoons reserved for propagation purposes greatly exceeded requirements.

Production costs were somewhat higher than in the two previous years. The cost of labor was $10,281. Of this, $7,343 was for general assistance and $2,938 was the salary of the insectary superintendent. There were 3,082 sacks of potatoes used at a cost of $5,456.

PRACTICAL RESULTS

As pointed out originally, this project had two objectives: first, to develop a cheap method for mass production of the parasite *Macrocentrus ancylivorus*, anticipating the time when orchard control of the oriental fruit moth would become necessary; second, to retard as long as possible the spread of the oriental fruit moth into the great commercial peach-producing areas of the state.

TABLE 1

COLONIZATION OF *Macrocentrus ancylivorus*, BY MONTHS

Month	Number of parasites colonized		
	Year 1944	Year 1945	Year 1946
	thousands	*thousands*	*thousands*
April	103	736	1,539
May	1,171	4,160	3,547
June	3,965	3,110	3,562
July	4,537	453	3,901
August	3,559	1,754	4,300
September	3,695	3,213	4,296
October	2,265	2,509	1,790
Total	19,295	15,935	22,935*

* The total number produced in 1946 was 28,986,000.

It is generally agreed that the first objective has been satisfactorily attained. It is difficult, however, to measure the degree to which the second objective has been reached. Aside from a few backyard trees in Orange County, the pest has not, up to this time, built up appreciably in the state. The extent to which the condition can be attributed to *Macrocentrus* is extremely difficult to determine, because of the scarcity of larvae of the host. The only larvae found in the commercial peach-growing areas in 1945 and consequently the only data available bearing on this point are given in table 3, compiled by Leslie M. Smith, Division of Entomology and Parasitology, University of California, from data collected by Frank Summers at Parlier, Fresno County.

TABLE 2

COLONIZATION OF *Macrocentrus ancylivorus*, BY COUNTIES

County	Number of parasites colonized		
	Year 1944	Year 1945	Year 1946
	thousands	*thousands*	*thousands*
Fresno	899	2,766	6,490
Kern	3,287	2,216	2,251
Merced	......	1,610	3,572
San Bernardino	1,987	1,531	1,729
Kings	1,440	1,427	3,121
Tulare	4,080	1,394	1,415
Sutter	3,291	1,317	......
Riverside	757	1,001	781
Stanislaus	2,412	886	1,335
Orange	......	839	214
Los Angeles	1,142	812	356
Santa Clara	......	75	651
Placer	......	41	383
Sacramento	......	20	637
Total	19,295	15,935	22,935

TABLE 3

PARASITISM IN LARVAE COLLECTED FROM PEACH TWIGS AT PARLIER*

Date collected and orchard	Larvae in sample	Larvae parasitized by *Macrocentrus*	Larvae not parasitized
1945: July 26, Bigger	3	2	1
Aug. 13, Bigger	4	1	3
Aug. 14, Slaiker	4	2	2
Aug. 16, Slaiker	1	1	0
Aug. 20, Barr	6	6	0
Aug. 21, Barr	5	5	0
Sept. 21, Bigger	2	1	1
Oct. 1, Barr	2	1	1
Oct. 4, Barr	1	0	1
Total, 1945	28	19	9
1946: June 11, Bigger	3	3	0
June 12, Bigger	6	4	2
June 17, Bigger	7	7	0
June 19, Bigger	1	1	0
July 1, Bigger	18	5	13
July 3, Barr	1	0	1
July 3, Bigger	18	8	10
July 7, Bigger	15	8	7
July 22, Bigger	4	4	0
July 30, Bigger	4	1	3
Aug. 6, Bigger	16	12	4
Aug. 7, Bigger	26	19	7
Aug. 12, Bigger	6	4	2
Aug. 15, Barr	3	2	1
Aug. 21, Bigger	1	0	1
Total, 1946	129	78	51

* Data supplied by Francis Summers, through Leslie M. Smith, Division of Entomology and Parasitology.

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Several staff members in addition to the authors made important contributions in the development of methods. Blair Bartlett was in charge of the insectary at Albany from its inception to July, 1945, and was responsible for initiating the development of the process now used for freeing parasite cocoons from moth cocoons by use of a silk solvent. He also made major improvements in the design of moth oviposition units. C. H. Martin was instrumental in refining several techniques involved in parasite production as a result of detailed researches at Albany. F. A. Gunther suggested mixing carbon dioxide with ether to render the latter nonexplosive. James K. Holloway, for several years engaged in *Macrocentrus* and other parasite work for the United States Bureau of Entomology and Plant Quarantine in the eastern United States, was of much assistance as a result of his extensive experience, and he was responsible for the detailed arrangements for field colonization of the parasite by the California State Department of Agriculture and the county agricultural commissioners.

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The late David B. Mackie, of the California State Department of Agriculture, gave enthusiastic support to the project, as has his successor, H. M. Armitage. The original stock of *Macrocentrus* was supplied by H. W. Allen, of the United States Bureau of Entomology and Plant Quarantine. L. A. Burtch, Agricultural Commissioner of Kern County, and J. S. Rowell, Agricultural Inspector, Santa Barbara County, were of material assistance in arranging for purchase of potatoes.

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RATE OF PIGMENT DEGRADATION IN THE PHLOEM OF DEHYDRATED RED CORE CHANTENAY CARROTS[1]

ELLIOT WEIER[2]

INTRODUCTION

THE DETERIORATION of dehydrated carrots in storage involves complex reactions. Although detailed information about these changes is not available at present, the following general facts are known: (1) the pigments present disappear; (2) haylike and other off-odors develop; (3) changes in the oil droplets within the cells result in a positive test for aldehydes with Schiff's reagent; (4) the carrots darken (apparently because the amino acids combine with sugars), and the substance responsible for this change in color is water-soluble; (5) blanching inactivates enzymes and also modifies other changes that occur in storage; (6) sulphite preserves color, especially by preventing the darkening reaction and by acting somewhat as an antioxidant; (7) other changes unquestionably occur.

In preliminary studies on carrots during processing and storage, the over-all changes in pigment concentration were expected to serve as a marker for at least some of the oxidations taking place in the lipid phase. This assumption was based on the following facts: (1) concentration of pigment may change greatly in storage; (2) carotene is a pro-oxidant (Olcott and Mattill, 1936*a*)[3] and its breakdown in oil solution results from the previous oxidation of the oil (Strain, 1941; and Sumner, 1942); (3) carotene in blanched and in dried carrots is dissolved in oil droplets (Weier, 1944*a*).

This paper reports experiments to test the stability of the pigment complex in carrots after various treatments. These treatments include blanching, leaching, and soaking in buffers, in solutions of high and low pH, in organic and mineral acids, and in antioxidants. Pigment concentration has been measured on the Evelyn colorimeter. Thus far, no attempt has been made to isolate individual pigments or to follow the details of their breakdown.

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[3] See "Literature Cited" for citations, which are referred to in the text by author and date.

METHODS

Phloem was secured from five plantings of Red Core Chantenay carrots. The carrots, which were grown at Davis, were harvested immediately before use. Seed was sown in April and September, 1943 (plantings 1 and 2); in April and September, 1944 (plantings 3 and 4); and in April, 1945 (planting 5).

The technique used was the same as that previously reported by Weier (1944*b*), except that more uniform samples were obtained by a careful distribution of carrot cylinders. That this greater uniformity produced good agreement between duplicate samples of the same experiment, and between similar experiments, is shown by figure 1. Curves 1 to 4 (fig. 1) are plots of carotene degradation in blanched carrot cylinders held at 60°C in moist air for the time intervals indicated. Curves 1 and 2 are taken from experiments run on October 16 and 18. Curve 4 is from an experiment of June 27. Curve 3 is the average of ten experiments on pigment breakdown in blanched, summer- and fall-harvested carrots. The greatest difference obtained in these experiments is shown by curves 1 and 4. Curve 5 represents the carotene degradation in unblanched carrot cylinders. The points obtained in this experiment are given on the graph; agreement was equally good in other curves.

For the experiments with dried carrots two types were used: (1) Carrots, cut into cubes approximately 1 cm on edge, were blanched, treated, and dried for 20 hours in a laboratory drying oven. They were then stored in glass jars at 40°C for 3 months, and the carotene was determined at 30-day intervals. The moisture content was about 7 per cent. (2) Carrots were blanched, treated, and dried in an experimental dehydrater made by members of the Agricultural Engineering Division. The drying time was 5 hours.

After drying, both types were ground to pass a 20-mesh screen. All powder small enough to pass a 48-mesh screen was sifted out. The moisture content averaged 5 per cent. For pigment determinations, boiling water was poured over a weighed sample of the powder and it was then allowed to stand for 1 hour. Water-soluble pigments that developed during storage were washed out. The carotinoid pigment was extracted in acetone. Determinations were made with an acetone extract in the Evelyn colorimeter. The usual precautions for protecting carotene solutions against light were observed. Most colorimetric examinations were made within 2 hours after extraction, and when this was not possible, solutions were held at 4°C until examined. All determinations were completed within 18 hours after extraction. In this 18-hour interval, no change occurred in the pigment held at 4°.

Dilutions of the pigment extracts showed a straight-line relation when examined on the Evelyn colorimeter, provided the galvanometer readings were kept between 30 and 70. The deviation from a straight line beyond 70 was considerable. Results were calculated in terms of the optical density of pigment per gram of fresh carrots. Since constants have been worked out with Eastman Kodak carotene (10 per cent A, 90 per cent B), the results could be expressed in micrograms of this sample. Such a procedure, however, seemed unwise, because neither the pigments of the carrot extract nor the detailed changes occurring in these pigments have been analyzed. Unquestionably, however, the density as measured by the Evelyn colorimeter represents concentration of pigments. To facilitate comparisons between experiments, results have been expressed on a percentage basis.

RESULTS

Age and Season. Carotene content varies with age and season. Planting 3 was sampled over a period of 10 months; other plantings for shorter periods. The first carrots selected for experimentation in planting 3 were 100 days old. Expressed in terms of Eastman carotene, the phloem in these carrots contained about 88 micrograms of carotene per gram of fresh carrot. During the next 30 days, the amount of pigment doubled to 176 micrograms per gram of fresh carrot phloem. This concentration remanied fairly constant from the middle of July to the middle of October. The amount then increased rather rapidly to 264 micrograms, which remained constant for the remainder of the experiment. Higher figures were occasionally obtained, the highest being 320 micrograms in a late-October experiment. Judging from the evidence, the pigment of California-grown carrots increases slowly for as long a time as 7 months, and does not remain constant after 130 days, as Barnes (1936) reports for carrots grown in New York. There is no evidence that the amount of carotene decreases during the California rainy season.

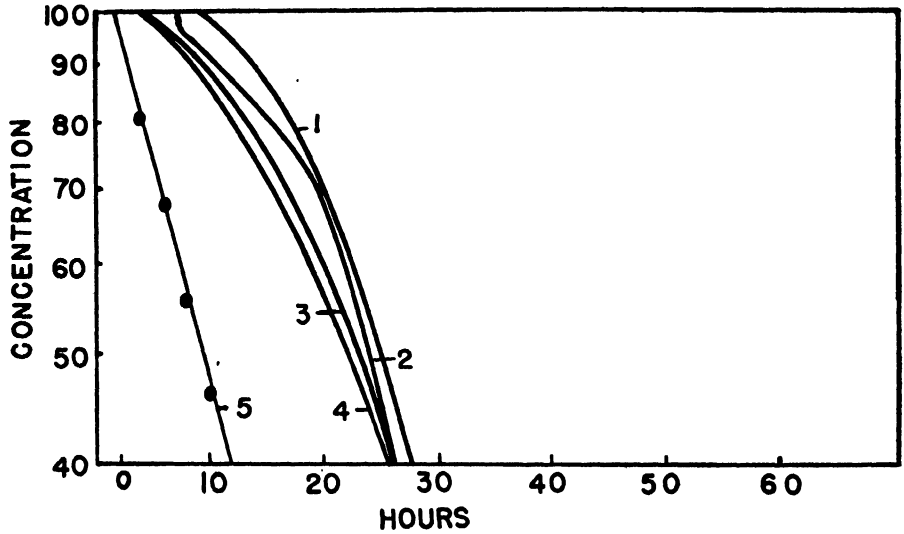

Fig.1. Rate of carotene breakdown in the phloem of summer- and fall-harvested carrots: curves 1, 2, and 4, separate experiments on blanched phloem; curve 3, average of 10 summer and fall experiments on blanched phloem; curve 5, summer experiment on unblanched phloem.

Unblanched Carrots. Pigment concentration of fresh carrots plotted against time on semilog paper results in a straight line (fig. 1, curve 5) and therefore follows a first-order reaction. Zero time was determined by extrapolation of the curve to 100 per cent carotene. The *k* value[4] for the curve shown was 10.7 at 4 hours; it remained practically constant over 30 hours, varying only from 9.05 to 9.88.

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[4] In calculating *k* from the expression $\left(\frac{1}{t}\log\frac{a}{2-x}\right)$, the time is in hours, the logarithm to base *e*.

The rate of pigment breakdown in unblanched carrots varied with the season. This variation is shown in table 1. The pigment was least stable in the fall, as is indicated by a high value for *k*. Its stability increased during the winter, and as the carrots aged. The rate of pigment degradation was most rapid by the end of 4 hours' incubation in all experiments except one—that carried out on April 9, when no breakdown had occurred at 4 hours.

In sample 4 (planted in September, 1944) the rate of pigment disappearance agreed closely with that noted in the older sample when the roots of both samples were dormant (March 15). When spring growth was resumed (March 22), however, the pigment was much less stable in the younger roots than in the older ones. The spring season was such that both plantings bolted upon the resumption of growth. The carrots sown in September of the previous year (planting 2) had grown vegetatively several months before bolting. Although rate studies were not carried out on this planting, the figures available indicate a rapid breakdown of the pigment in spring-harvested carrots.

TABLE 1

RATE OF CAROTENE BREAKDOWN, AS INDICATED BY THE SPECIFIC RATE CONSTANT *k*, IN UNBLANCHED CARROTS HARVESTED AT DIFFERENT SEASONS AND INCUBATED AT 60° C IN MOIST AIR

Hours	Planted 1943		Planting 3, planted April, 1944						Planting 4, planted September, 1944		
	Feb. 9, 1944	Feb. 10, 1944	Sept. 15, 1944	Oct. 23, 1944	Nov. 14, 1944	Dec. 15, 1944	Feb. 2, 1945	April 9, 1945	March 15, 1945	March 22, 1945	April 23, 1945
	k	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*
4	......	......	. .	10.6	...	. .	3.8	0	...	...	...
6	......	......	3.2	9.7	6.7	5.6	...	...	...	. .	...
8	......	.....	3.1	9.9	6.5	4.8	5.8	2.1	2.4	7.4	6.4
21	4.4	4.0	3.1	9.7	4.7	4.7	3.9	1.9	1.7	6.5	5.7
26	......	......	4.7	9.5	4.6	4.6	3.1	2.0	1.4	5.4	10.0

A series of four experiments run during February, 1944, in which unblanched carrots were treated like those studied in February, 1945, gave the following values for *k* after 21 hours' incubation: 3.95, 4.37, 4.23, and 3.95.

Blanched Carrots. Season also affected the stability of carotene in blanched undried carrots. During a given season, however, the stability of the carotene was constant. Good agreement was obtained between experiments on a single culture carried out during the summer and fall of 1944 (fig. 1, curves 1 to 4). In winter and spring the pigment in the roots of this same planting was more stable (fig. 2, planting 3, winter). The agreement among nine experiments was good; the curve shown in figure 2 is the average. Furthermore, different plantings harvested in the same season of different years agreed well in rate of carotene breakdown (fig. 2). There is one exception—compare spring-harvested carrots of plantings 2 and 4. These carrots were planted in September of 1943 and 1944, respectively, and harvested in the springs of 1944 and 1945. Planting 2, however, had a long growing season in the spring of 1944, whereas planting 4 bolted immediately upon the resumption of growth. Pigment broke down rapidly in the growing, spring-harvested carrots of planting 2, but remained stable in the bolting, spring-harvested carrots of planting 4. Planting 5 was sown in April, 1945. When first examined in July, the roots were growing vigorously. The results (fig. 2, summer, 5) agree closely with those obtained for other summer-harvested roots.

In eighteen out of twenty experiments on blanched carrots, the rate of pigment breakdown was slow at the start of the experiment, but increased at longer incubation periods. The two exceptions were obtained with winter-harvested roots. Values for *k*, calculated as the constant for a first-order reaction during the summer, ranged from 0.00 at 4 and 8 hours to 3.80 at 26 hours. In winter the values of *k* ranged from 0.00 at 4 and 8 hours to approximately 0.48 at 26 hours.

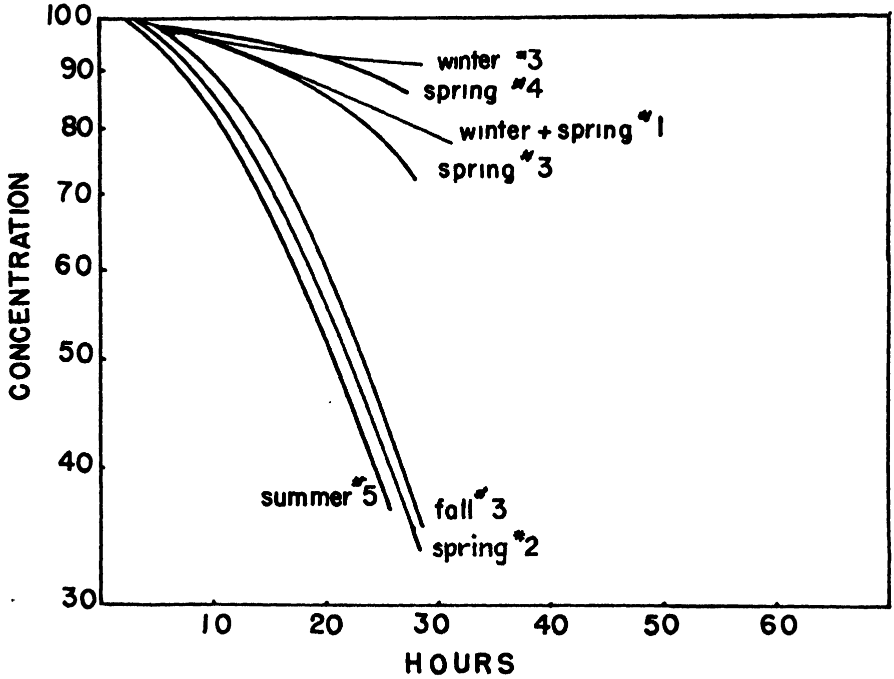

Fig. 2. Rate of carotene breakdown in blanched cylinders of carrot phloem. Seasons are indicated. Numbers refer to planting of carrots.

Judging from the course of the reaction as shown by these results, the summer-harvested carrots contained an inhibitor, which slowed up the reaction during the first 8 hours of incubation. As the inhibitor was destroyed, the rate of pigment degradation increased.

Leaching Blanched Undried Carrots. It was previously reported (Weier, 1944*b*) that the stability of carotene is lowered if the carrot dice are washed after blanching, and that this is more apparent in winter than in summer. These results have been corroborated and extended (fig. 3). Curves 1 and 1 leach (fig. 3) show the difference in rate of carotene breakdown in blanched dice (summer-harvested) when washed after blanching (curve 1 leach) and when not so washed (curve 1). Similarly, curves 2 and 2 leach show the rate of pigment breakdown for winter-harvested carrots. Carrots were washed in running water for 20 minutes immediately after blanching. The difference between washed and unwashed dice was several times greater in winter-harvested than in summer-harvested carrots. Carotene was more stable in winter-harvested carrots, however, even after washing, than in unwashed summer carrots.

Leaching Blanched Dehydrated Carrots. Washing after blanching lessened the stability of the pigment in dried blanched carrots—a fact illustrated by the curves in figure 4. In this series of experiments, carrots were diced and blanched. After one half of the dice had been soaked in water for 20 minutes, both samples were dried and stored at 40° C for 3 months. Analyses were made every 30 days.

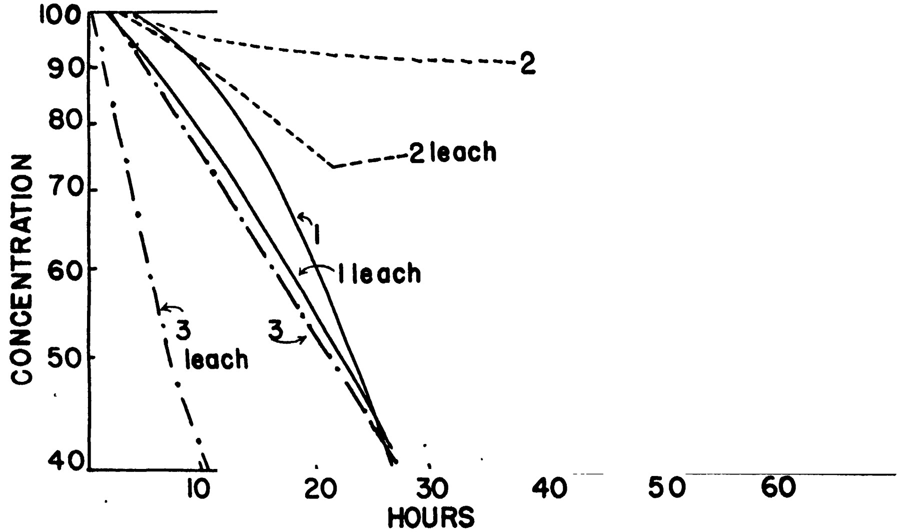

Fig. 3. Rate of carotene breakdown in carrot phloem washed after blanching, as contrasted with unwashed blanched carrot phloem. Curves 1 and 1 leach, summer-harvested carrots, 1944. Curves 2 and 2 leach, winter-harvested carrots, 1944, 1945. Curves 3 and 3 leach, spring-harvested dried carrots, ground, stored in oxygen at 60° C, and analyzed at the hours indicated.

In seven out of eleven experiments on blanched washed carrots carried out between July 7 and October 31, the specific rate constant varied but slightly during the course of the experiment. It varied, for example, from 2.34 to 2.72; from 2.02 to 2.36; and from 2.48 to 2.52. In the other four experiments it increased during the course of the experiment—for example, from 1.04 to 2.42 and from 1.41 to 2.74. Although this indicates some retardation in the rate of breakdown during the initial hours of the experiment, the retardation is less than in the unwashed blanched carrots. (Compare 1.04 to 2.42 for blanched washed carrots with 0.00 to 3.80 for blanched unwashed carrots.) In these latter four experiments it may be assumed that the inhibitor was not completely removed by the leaching.

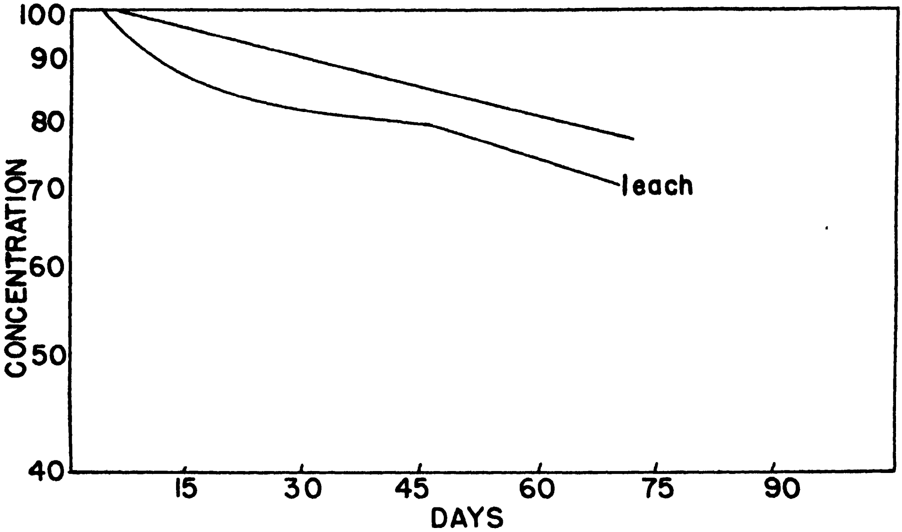

Fig. 4. Rate of carotene breakdown in winter-harvested dehydrated carrots stored at 40° C and analyzed monthly.

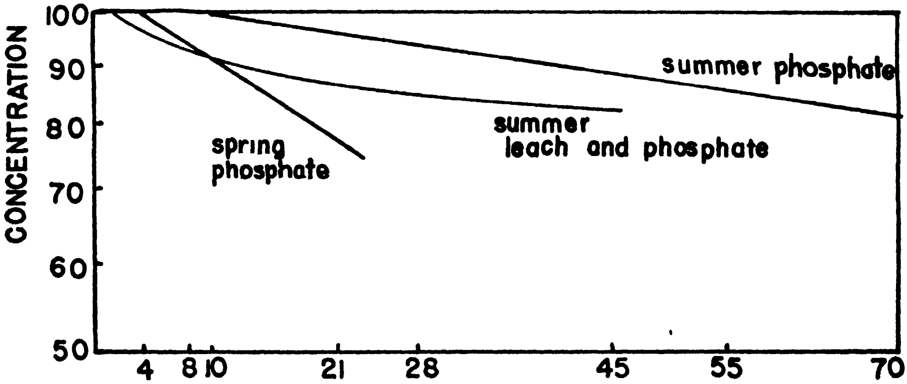

Fig. 5. Rate of carotene breakdown in cylinders of summer-harvested, blanched carrot phloem that was impregnated with standard phosphate buffer at pH 7.1.

In still another series of experiments, carrots were blanched; one half of the sample was leached in distilled water. They were dried and ground. These samples were incubated over oxygen at 60° C. The results are shown in curves 3 and 3 leach (fig. 3). The carotene in the leached sample broke down more rapidly than that in the blanched, unleached sample.

The evidence obtained indicates that when blanched carrots are washed, some substance that acts to retard the breakdown of pigment is removed.

Buffer Treatments—Undried Carrots. Pigments in summer-harvested carrots that had been blanched and treated with phosphate buffers close to neutrality, were stable when incubated at 60° C in moist air (fig. 5, summer). The pigments in summer-harvested carrots that were leached after blanching and then treated with neutral phosphate buffer were stable (fig. 5, summer, leach and phosphate). In these experiments the blanched carrots were soaked in the buffer for 20 minutes and were kept moistened with it during incubation. In the tests that furnished the data for the spring curve of figure 5, the blanched dice were soaked for 20 minutes in the buffer. During incubation the dice were moistened with distilled water; from these, soluble phosphorus compounds diffused during incubation (fig. 7). The results indicate that the breakdown of pigment was retarded somewhat by soaking the dice in neutral phosphate buffers before incubation. (Compare fig. 5 with fig. 2, summer and fall curves.)

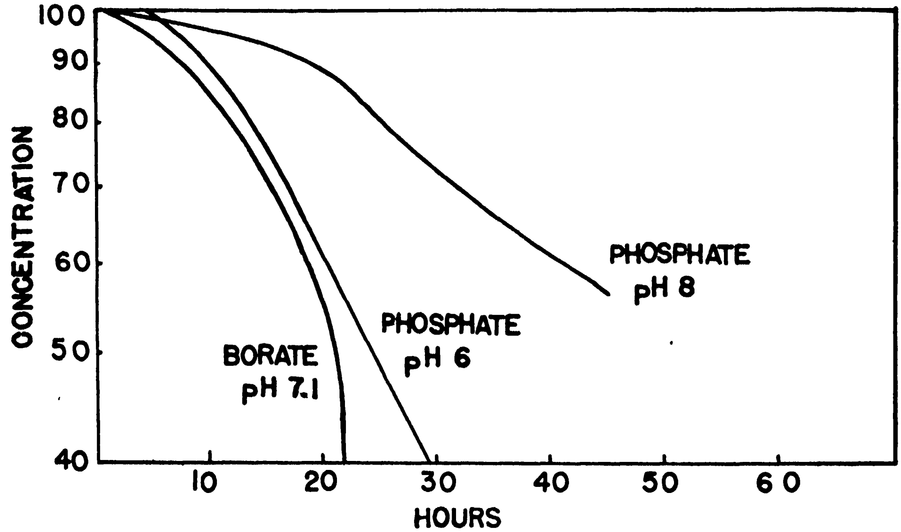

Fig. 6. Rate of carotene breakdown in cylinders of blanched carrot phloem, impregnated with standard phosphate buffers at pH 8 and 6 and standard borate buffer at pH 7.1.

A neutral borate buffer did not stabilize the carotene. Blanched dice were soaked in the buffer for 20 minutes and kept moistened with it during incubation in moist air at 60° C (fig. 6, borate).

Acid and alkaline phosphate buffers differ in their protective properties. In blanched dice that were soaked in these buffers and moistened with them during incubation at 60° C, the pigment was degraded more slowly than in unphosphated blanched dice. (Compare fig. 6 and the curves for summer-harvested carrots of fig. 2.) Alkaline phosphate buffers protected the carotene much more than did the acid phosphate buffers. Neither acid nor alkaline phosphate buffers stabilized the pigment so well as the neutral phosphate buffer (fig. 5).

Buffer Treatments—Dehydrated Carrots. Results of phosphate treatments of dried carrots are at variance with each other. Winter-harvested carrots were diced and blanched. They were treated in vacuum for 5 minutes, with dilute solutions of NaH_2PO_4 and Na_2HPO_4 and standard buffer solutions at neutrality and at pH 8. The dice were dried and stored in air at 40° C for 3 months. Carotene determinations were made monthly. Pigment degradation was faster in the phosphated dice than in the control blanched dice. After 3 months' storage, blanched dehydrated carrot dice still retained 83 per cent of their original carotene, whereas phosphated dice contained only 74 per cent.[5]

Leaching of Soluble Phosphates. Leaching of soluble phosphate was tested under the experimental conditions (fig. 7). Phosphorus determinations were made according to the method described by Carolus (1938). In raw, untreated carrots the phosphate diffused very rapidly. About 10 per cent of the soluble phosphorus was lost in blanching, and another 40 per cent in leaching. Phosphorus continued to diffuse from all carrot dice during incubation. Its rapid disappearance from untreated dice and its low value in the dice after washing could account for the greatly increased carotene breakdown in these carrots. (Compare fig. 6 with the previous figures.)

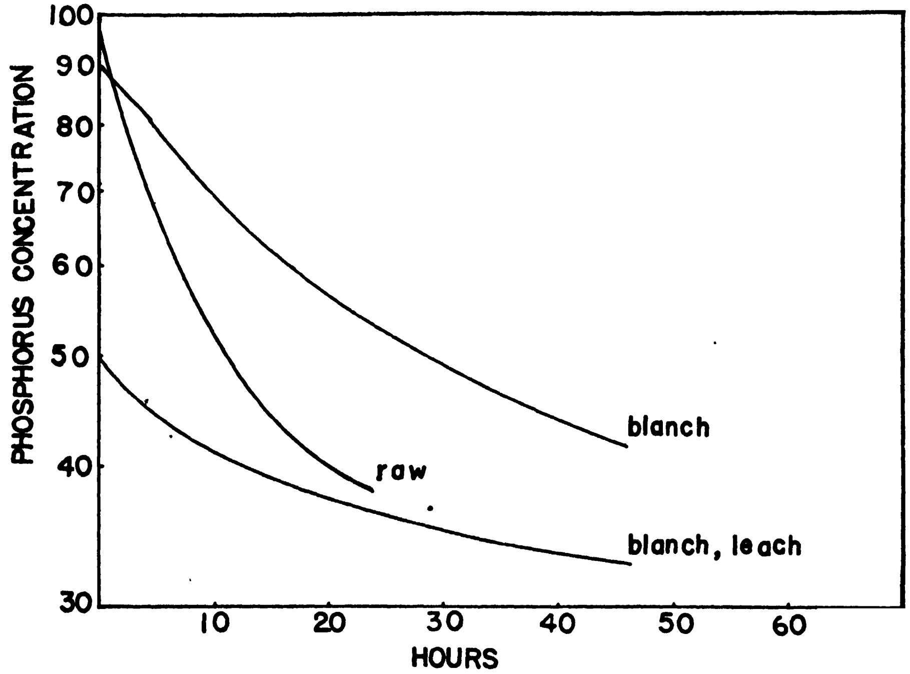

Fig. 7. Rate of diffusion of soluble phosphate from raw, blanched, and blanched leached cylinders of carrot phloem.

Blanched dice soaked in phosphate buffer contained at least three times the normal amount of phosphorus. Conceivably, phosphate may retard the breakdown of the carotene during the initial stages of the reaction. Phosphates and phosphoric acid are known to be antioxidants (Eckey, 1932).

Organic Solvents—Undried Carrots. Many antioxidants are fat-soluble, and in order to impregnate blanched carrot dice with them, a solvent must be used that will carry the antioxidant but will not remove the pigments. The stability of the pigments was tested after shaking the diced carrots in alcohols. These experiments were made with winter-harvested carrots, in which, because of the stability of the pigments, the effect of the alcohols on the inhibitor system could also be observed. The carotenoids are virtually insoluble in the concentrations of alcohols used; but small amounts of pigment were washed from the dice during the immersion in the alcohols.

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[5] These carrots were stored in an oven without forced draft. Localized convection currents over prolonged periods of incubation may account for a 100 per cent difference in paired samples, especially since the oven had to be crowded.

Some blanched carrot dice were shaken for 30 minutes in 70 per cent ethyl alcohol, and others in 40 per cent methyl alcohol. All were then washed in several rinses of distilled water to remove as much alcohol as possible. During incubation they were moistened with distilled water. The breakdown of the pigment was comparable with the breakdown in dice that were washed in running water (fig. 8).

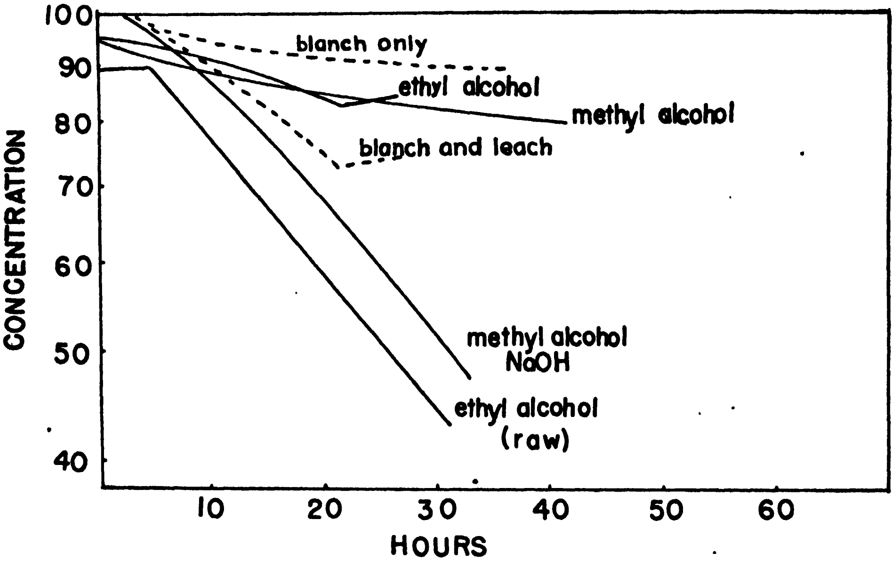

Fig. 8. Rate of carotene breakdown in blanched cylinders of winter-harvested carrots treated with alcohol after blanching. Dotted lines show the rate of carotene breakdown in blanched and blanched leached, winter-harvested carrots.

Raw dice infiltrated with 70 per cent ethyl alcohol were rinsed several times in distilled water and were moistened with it during incubation; see fig. 8, curve labeled "ethyl alcohol (raw)." A control experiment with untreated dice (curve not included in fig. 8), run concurrently, showed good agreement. The curves coincide at 4 and at 26 hours. The alcohol-infiltrated dice contained 10 per cent more pigment at 8 and 21 hours.

Blanched dice were infiltrated with 10 per cent NaOH in 40 per cent methyl alcohol. They were then washed, and were kept moistened with distilled water during incubation. The pigment decomposed very rapidly (fig. 8). Alkaline methyl alcohol must, in some way, destroy the inhibitor system that is present as a result of blanching.

Mineral Acids and Bases. The stability of the pigment in winter-harvested carrots facilitates a study of the influence of various substances on the in-

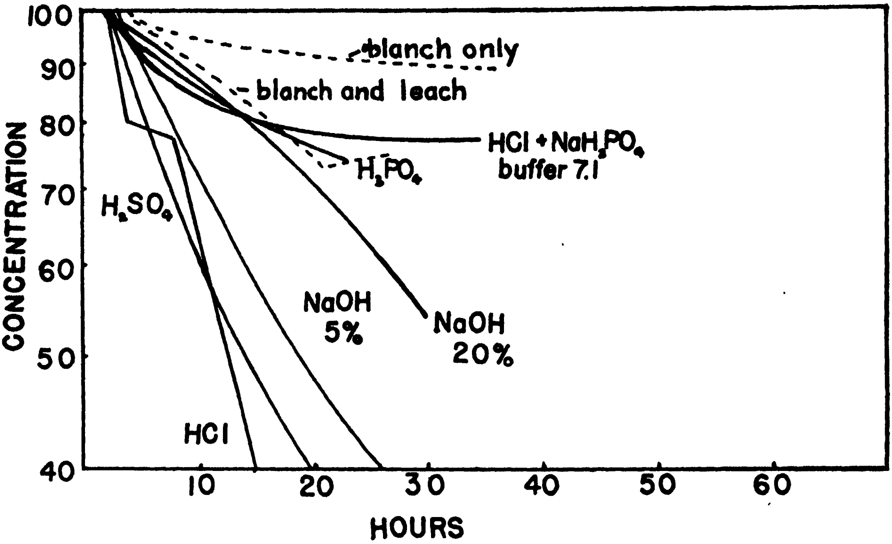

Fig. 9. Rate of carotene breakdown in blanched cylinders of winter-harvested carrots treated with inorganic acids after blanching. Dotted lines show the rate of carotene breakdown in blanched and blanched leached winter-harvested carrots.

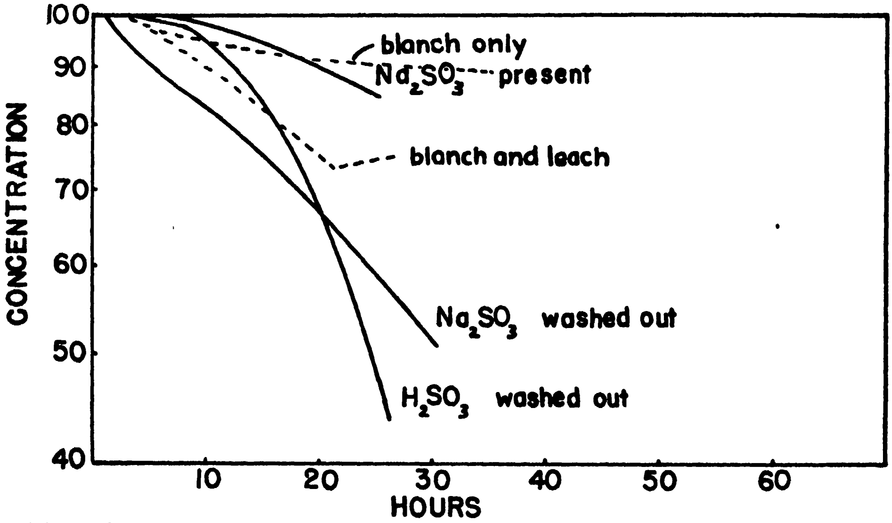

Fig. 10. Rate of carotene breakdown in blanched cylinders of winter-harvested carrots treated with SO_2 after blanching. Dotted lines show the rate of carotene breakdown in blanched and blanched leached winter-harvested carrots.

hibitor system, which is released or activated by blanching. Blanched dice of winter-harvested carrots were soaked in aqueous solutions of the following materials: 5 and 20 per cent NaOH; approximately 0.5 molar H_2SO_4; H_2SO_3 to give approximately 1 part SO_2 in 1,000 parts water; approximately 1 molar HCl; 0.5 per cent Na_2SO_3; and approximately 0.5 molar H_3PO_4. In all treatments except with one Na_2SO_3, the blanched dice were washed after soaking and were kept moist with distilled water throughout incubation. The results of these experiments are shown in figures 9 and 10. The presence of Na_2SO_3 during incubation protects the pigment (fig. 10, Na_2SO_3 present).

Pretreatment with H_3PO_4 and 20 per cent NaOH apparently does not affect the inhibitor system. The rate of pigment degradation compared well with the rate of breakdown in blanched and washed carrots (fig. 9).

Pigment decomposition was accelerated by soaking blanched dice previous to incubation in H_2SO_4, H_2SO_3, Na_2SO_3, HCl, and 5 per cent NaOH, when these were not present during incubation (figs. 9 and 10). The action of the inhibitor system present in the dice after blanching was, in some manner, destroyed or hampered by these substances.

Dice blanched and treated with HCl were washed in distilled water and soaked in neutral buffer solution. They were moistened with buffer throughout incubation. Pigment degradation was comparable with that of blanched and washed winter-harvested carrots (fig. 9, HCl + NaH_2PO_4 buffer 7.1).

Organic Acids—Undried Carrots. Blanched dice of winter-harvested carrots were shaken for 30 minutes in solutions of various organic acids (Olcott and Mattill, 1936*b*). They were then drained and separated into two groups. One group (curves numbered 1 in figs. 10 and 11) was moistened with the acid during incubation. The second group (curves numbered 2 in figs. 11 and 12) was rinsed in distilled water after treatment with the acid and was moistened with distilled water during incubation. This procedure allowed the acid to diffuse from the dice, so that it was possible to differentiate between an interference with the action of the inhibitor and a stabilization of the pigment by the acid. The following organic acids were used: 0.1 molar oxalic acid; 0.25 molar maleic acid; nordihydroguaiaretic acid, 1 part in 3,500 parts 6 per cent ethyl alcohol; 0.1 per cent gallic acid; 0.1 per cent ascorbic acid; and sodium salts of oxalic and maleic acids adjusted to neutrality by the addition of NaOH.

Treatment with the sodium salts of oxalic and maleic acids had no beneficial effect (fig. 11). The results agreed well with those obtained from experiments on blanched dice of winter-harvested carrots. Soaking and incubation with sodium maleate greatly accelerated the degradation of the pigment.

Treatment with nordihydroguaiaretic acid, before and during incubation, markedly decreased the decomposition of the pigment (NDGA 1, fig. 12). When the nordihydroguaiaretic acid was washed out before incubation, the pigment was degraded more slowly than in blanched and washed winter-harvested carrots (NDGA 2, fig. 12). Treatment with oxalic acid likewise appeared to protect the carotene, the results being about the same whether it was applied during incubation or only before (oxalic, 1 and 2, fig. 12). Gallic acid and ascorbic acid, when present during incubation, also protected the pigments. Washing after treatment with gallic and ascorbic acids gave results that approached those of blanched and washed winter-harvested carrots. Maleic acid, when present during incubation, produced similar results. Breakdown was always more rapid in the dice that were washed after infiltration with the acid than in those that remained unwashed. When maleic acid was washed from the dice and the carrots were moistened with distilled water during incubation, the breakdown was very rapid.

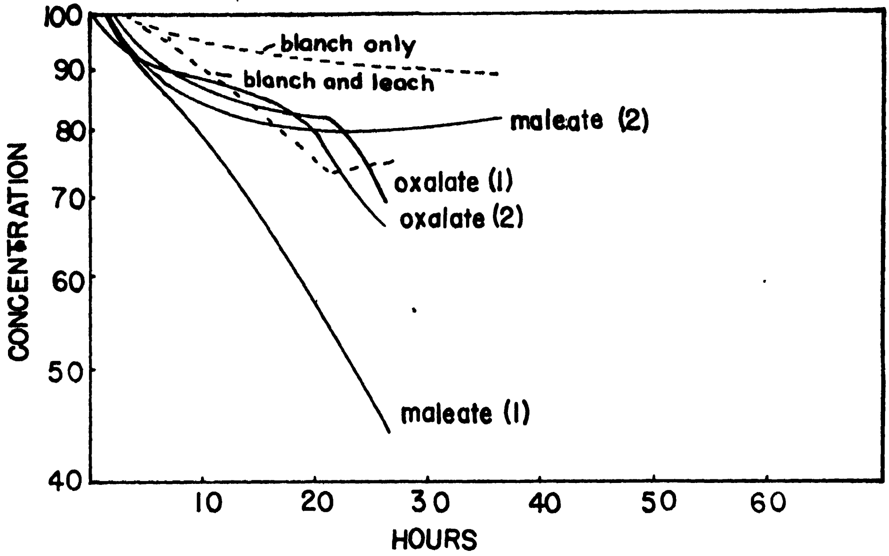

Fig. 11. Rate of carotene breakdown in blanched cylinders of winter-harvested carrots treated with sodium salts of maleic and oxalic acids after blanching and moistened with the salt (1) or with distilled water (2) during incubation. Dotted lines show the rate of carotene breakdown in blanched and blanched leached winter-harvested carrots.

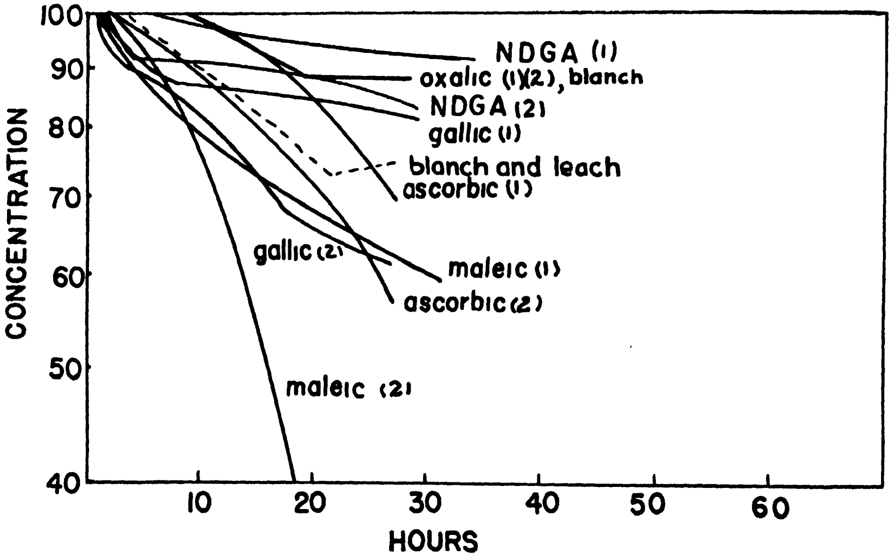

Fig. 12. Rate of carotene breakdown in blanched cylinders of winter-harvested carrots treated with dilute organic acids after blanching and moistened with the acid (1) or rinsed and moistened with distilled water (2) during incubation. Dotted line shows the rate of carotene breakdown in blanched leached winter-harvested carrots. The curve for the blanch experiments corresponds to the curve for the oxalic acid treatments.

Organic Acids—Dehydrated Carrots. In another series of experiments, dice were soaked in these organic acids for 30 minutes. They were then dried to about 6 per cent moisture and stored at 40° C in air for 3 months. Carotene determinations were made monthly (fig. 13).

In dried carrots infiltrated with nordihydroguaiaretic or dipped in ascorbic acid after blanching, the pigment was very stable (NDGA and ascorbic dip, fig. 13). Soaking blanched carrot dice in ascorbic acid for 30 minutes under vacuum appeared to prolong the induction period; but breakdown of pigment was rapid once it had started (ascorbic aspirate, 30 min., fig. 13). Maleic acid also appeared to prolong the induction period. The pigment broke down rapidly after the treatment with oxalic acid (fig. 13).

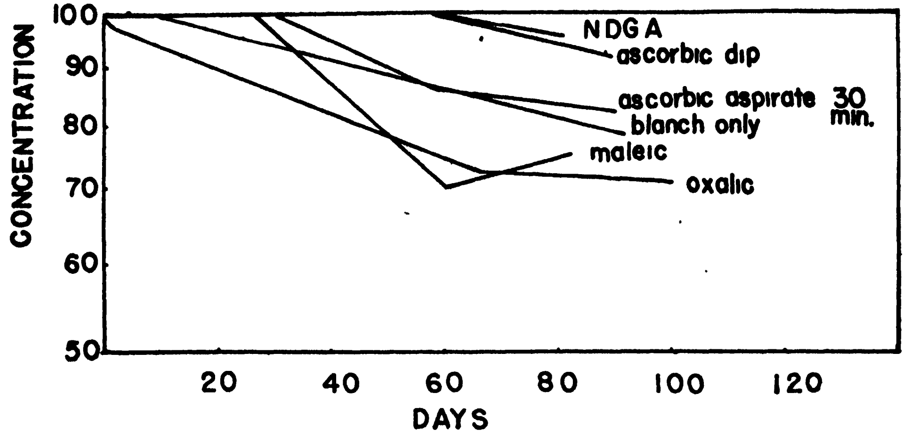

Fig. 13. Rate of carotene breakdown in diced, blanched, dried carrots treated with antioxidants after blanching and stored at 40° C for 3 months.

DISCUSSION

Quality deterioration of dehydrated carrots appears to have at least two separate causes: deterioration in an oil-pigment fraction, and reactions in an aqueous fraction. Thus far, changes in the aqueous fraction have been considered only in a general way.

Results indicate that pigment breakdown may be stabilized by antioxidants. These results, however, are only preliminary; they need verification on other varieties of carrots grown in other localities. Spectrometric studies on pigment degradation might well revise the findings reported here. The investigations on rancidity changes in vegetable oils suggest other combinations of antioxidants that might improve the keeping qualities of dehydrated carrots. Some of these possibilities are being studied.

The present data indicate that antioxidants may be helpful in preserving the quality of dehydrated foods. Tressler and Du Bois (1944) and Bauernfeind and Siemers (1945) have shown that ascorbic acid, acting as an antioxidant, will prevent browning in peaches. According to Mills and Hart (1945), 0.9 per cent diphenylamine greatly retarded the breakdown of carotene in dehydrated oats and alfalfa.

SUMMARY

The rate of pigment breakdown in raw carrots incubated in moist air at 60° C was uniform. It sometimes decreased after prolonged storage.

The rate of pigment breakdown in blanched carrots incubated in moist air at 60° C indicated that an inhibitor of some kind retarded the rate of breakdown during the initial stages of the reaction.

The pigments in carrot roots were more stable in winter-harvested than in summer-harvested carrots.

Neutral phosphate buffers, nordihydroguaiaretic acid, Na_2SO_3, oxalic acid, gallic acid, and ascorbic acid, when present in the blanched undried carrots during incubation, retarded the rate of degradation of the pigment.

Nordihydroguaiaretic acid and ascorbic acid retarded the rate of pigment degradation in blanched and dried carrot dice.

HCl, H_2SO_4, H_2SO_3, and Na_2SO_8 destroyed the inhibitor system released by blanching. The pigment was degraded very rapidly when blanched dice were treated with these substances prior to incubation, when they were not present during incubation.

A substance that protects the carotene in carrot tissue after blanching may be leached from blanched dice by washing in running water.

ACKNOWLEDGMENT

Thanks are due to the Division of Truck Crops, to the members of the Division of Food Technology, and to Mrs. Marjorie Morris who did much of the technical work in this experiment.

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H I L G A R D I A

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AN EVALUATION OF NINE BACKCROSS-DERIVED WHEATS[1]

COIT A. SUNESON[2]

THE IMPROVEMENT of established varieties of wheat through use of the backcross method of breeding was pioneered at the California Agricultural Experiment Station (Briggs, 1930).[3] It seems advisable to present a new appraisal of the method that is based upon recent progress. For the plant breeder, the farmer, and the miller, the principal concern involves the degree of similarity, or difference, between a known commercial variety and its improved successor evolved by backcrossing where injury from diseases and insects does not occur. The results of numerous comparisons under such conditions are presented here. The procedures followed in the breeding of nine improved varieties and the genetic nature of these varieties are also discussed.

REVIEW OF LITERATURE

Theoretical assumptions regarding homozygosity of backcrossed populations and their similarity to their prototype varieties have been presented by Briggs (1930, 1938). These assumptions furnished the justification for the release of the improved varieties for commercial culture before extensive comparative performance data were available. Subsequently, a series of reports, each treating a successively larger body of data have been presented (Briggs, 1938; Suneson and Briggs, 1941; Suneson, Riddle, and Briggs, 1941; Riddle and Baker, 1944) emphasizing the similarity between the improved varieties and their prototypes. The improved varieties have been widely accepted by California farmers because of the economic advantage of the obvious improvements. In 1939, Baart and White Federation occupied 56 per cent of the California wheat acreage (Clark, 1942). In 1944, however, Baart 38 and White Federation 38, together with a very small residue of their prototypes, occupied approximately 70 per cent of the California wheat acreage, according to all available reports. This trend had been predicted (Suneson, 1945), but it was accelerated by state-wide rust damage in 1940 and 1941.

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[1] Compiled from investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Dept. of Agriculture; and the agricultural experiment stations of Arizona, California, Colorado, Idaho, Montana, Oregon, Washington, and Utah coöperating in a regional wheat-improvement program.

[2] Agronomist and Regional Coördinator, United States Department of Agriculture, and Associate in Agronomy, University of California.

[3] See "Literature Cited" for citations, which are referred to in the text by author and date.

MATERIALS AND METHODS

Since the results given in this paper are practical attempts to show the degree of return to the recurrent parental type in California backcross-derived varieties, special interest attaches to the pedigrees of the several improved varieties. The number of backcrosses and the size and management of the successive backcross generations are especially important. The pertinent facts are given below.

Big Club 37, C.I. No. 11901: Big Club 37 was derived from Martin backcrossed to Big Club six times (Martin x Big Club^7). The first cross was made in 1922 and six backcrosses were added and successively tested for bunt, generally on F_3 strains in alternate generations of backcrossing, until 1936, when 77 homozygous bunt-resistant F_3 lines were bulked to make the variety.

Sonora 37, C.I. 11902 (Martin x Sonora^7) : Sonora 37 was produced in the same way and at the same time, 71 F_3 lines being bulked.

Pacific Bluestem 37, C.I. 11903 (Martin x Pacific Bluestem^7) : This variety, also produced at the same time and in the same way, consists of a composite of 78 F_3 lines.

White Federation 38, C.I. 11906 (Hope-White Federation^5 x Martin-White Federation^6) : Martin smut resistance and Hope stem-rust resistance carried through independent backcrossing programs and then merged in a final cross from which 182 doubly resistant F_3 lines were bulked in 1937.

Baart 38, C.I. 11907 (Hope-Baart^5 x Martin-Baart^7) : This variety was produced in the same way and at the same time as White Federation 38, except that 157 F_3 lines were bulked.

Onas 41, C.I. 12,229 (Martin-White Federation^3 x Onas^6) : Onas 41 is a composite of 115 homozygous bunt-resistant F_3 lines.

Federation 41, C.I. 12,230 (Martin-White Federation^3 x Federation^6) : Federation 41 was produced concurrently with Onas 41 from a composite of 130 F_3 lines.

Poso 42, C.I. 12,237 (Dawson x Poso^6) : This Hessian-fly-resistant variety is a composite of 45 F_4 lines.

Big Club 43, C. I. 12,244: This variety is a composite of 144 triply resistant (bunt, Hessian fly, and stem rust) F_3 lines. It resulted from three independent breeding programs:

(1) Production of Big Club 37 as noted above.
(2) Dawson-Big Club^4 x Big Club 37^2.
(3) Hope-Baart^4 x Big Club^2 x Big Club 37^2.

The final merger involved random crossing of (2) on (3), and then of (2) on the F_1 of these.

Comparisons herein reported are drawn from plot or nursery tests in which the commercial variety and the improved strain were grown in paired tests, where stem rust, bunt, or Hessian fly was not reported as a differentiating yield factor. Most of the data were compiled from results at 18 experiment stations coöperating in the western regional wheat-improvement program (Suneson, Riddle, and Briggs, 1941). Other data came from coöperative tests on farms in California and Nevada. Yield data are reported for individual plots, while other data, such as height, heading date, test weight, and lodging are reported only as an average for the several replicates (either an average of independent measurements, or a single measurement on a composite sample). All available data on each item are included.

TABLE 1

COMPARATIVE YIELDS OF BAART AND BAART 38 AT WESTERN EXPERIMENT STATIONS AND IN CALIFORNIA AND NEVADA FARM TESTS, 1939-1944*

Station	Mean yields per acre		Yield differences	Binomial yield distributions		
				Number of times superior		Number of ties
	Baart	Baart 38		Baart	Baart 38	
	bushels	*bushels*	*bushels*			
California	35.9	36.6	+0.7	31	32	4
Nevada farms	29.1	29.9	+0.8	5	4	0
Mesa, Ariz.	38.8	39.3	+0 5	4	6	0
Sacaton, Ariz.	30.6	31.0	+0.4	1	2	0
Hesperus, Colo.	44.8	42.0	−2.8	3	2	1
Pendleton, Ore.	37.4	35.8	−1.6	11	7	0
Moro, Ore.	26.4	26.7	+0.3	16	14	0
Union, Ore.	54.2	55.3	+1.1	7	4	0
Pullman, Wash.	52.1	52.8	+0.7	9	13	2
Prosser, Wash.	49.9	48.4	−1.5	7	4	1
Lind, Wash.	25.1	25.3	+0.2	7	10	1
Pomeroy, Wash.	28.0	28.4	+0.4	5	4	2
Walla Walla, Wash.	38.3	38.4	+0.1	9	9	0
Moscow, Idaho	37.2	36.6	−0.6	6	12	0
Sandpoint, Idaho	24 8	24.6	−0.2	2	3	0
Tetonia, Idaho	28.4	25.1	−3.3†	16	6	0
Aberdeen, Idaho	68.4	68.6	+0.2	12	8	0
Bozeman, Mont.	58.6	57.3	−1.3	9	5	0
Logan, Utah	54.2	54.9	+0.7	7	6	0
All tests	39.59	39.45	−0.14	167	151	11

* This summarizes all coöperative plot and nursery tests reported to the author excluding only nonpaired tests, or tests wherein stem rust affected yields. The California tests include tests on farms in coöperation with the Agricultural Extension Service.

† Odds are greater than 19:1 that this difference is not due to chance.

Mean differences, supported by odds determined from the point binomial distributions (Miles, 1935) are used to determine the similarity between the commercial and the improved strain. This method of statistical analysis is conservative and is particularly useful in studies of this kind because of its ease of application.

RESULTS

Agronomic Evaluation. Since Baart was the most widely grown variety in the western region (Clark, 1942) and Baart 38 has now almost completely replaced it in California, Arizona, and Nevada and is being grown in Oregon, Idaho, and Utah, it is logical that the most extensive comparisons involve these two varieties. Detailed data on yield from all available sources in the region are presented in table 1. These represent tests from 1939 through 1944, excluding only nonpaired tests or comparisons in which stem rust was a differentiating yield factor. For the six-year period, 59 tests were excluded because of the presence of stem rust. Mean yields of the two varieties at all locations and the number of times when each was superior are shown. From this it can be deduced that only at Tetonia, Idaho, has a significant difference (at the 5 per cent level) between the two varieties been found. In this case the results favored Baart.

TABLE 2

DIFFERENCES IN AGRONOMIC CHARACTERISTICS BETWEEN BAART AND BAART 38, WESTERN REGIONAL TESTS*

Year	Date headed (April 16 to July 22)		Plant height (25 to 68 inches)		Lodging (0 to 25 per cent)		Weight per bushel (53.9 to 66.5 pounds)	
	Number of tests	Mean difference	Number of tests	Mean difference	Number of tests	Mean difference	Number of tests	Mean difference
		days		*inches*		*per cent*		*pounds*
1939	11	−0.1	11	−0.3	2	−9	13	+0.1
1940	10	+0.2	12	−0.3	6	+1	11	+0.3
1941	13	−0.8	12	−0.1	3	−2	4	+0.3
1942	11	0.0	12	−1.0	4	−6	12	+0.6
1943	10	+0.3	11	0.0	4	+5	11	+0.4
1944	10	+0.3	12	−0.5	5	−9	12	+0.3
Total or mean	65	−0.05	70	−0.36	24	−2.8	63	+0.33

* Exclusive of environments known to be favorable to the improved variety.

As an average of all 329 plot comparisons, no significant difference was found despite the wide range of environments in which the tests were conducted. This means that although Baart outyielded Baart 38 by an average of 0.14 bushel, there is little likelihood that there is any real difference between these two varieties.

Comparative agronomic data for Baart and Baart 38 are presented in table 2. It has been mentioned that the unit data treated here are available only as averages of all plots at each station. The fact that some stations have equipment for determining test weights that is calibrated in half-pound intervals, while others show 0.1 pound difference is also worthy of note. Nevertheless, the preponderance of individual instances in which Baart 38 was shorter and weighed more per bushel than Baart suggests that the small average differences reported are probably significant. The differences in height and in test weight were 0.36 inch and 0.33 pound per bushel, respectively. While Baart 38 showed a range of from 2 days earlier to 5 days later than Baart, its average heading date was the same as for Baart. Other differentiating agronomic comparisons failed to show significant differences between the two varieties. These comparisons are not shown in table 2, but such factors as shattering and susceptibility to mildew, septoria, stripe rust, and root rot are included.

In establishing some slight, but apparently significant differences between the two varieties, it must be remembered that they are nevertheless substantially alike—so much so that large populations and diverse environments must be sampled to establish the slight differences herein reported.

Apart from emphasizing similarities between Baart and Baart 38, it also seems desirable to call attention to the economic advantage resulting from the commercial distribution of Baart 38. Its resistance to certain races of bunt and stem rust is unquestioned. Comparative tests in the presence of bunt have not been conducted, but a fairly representative body of data from stem-rust environments is available. Of the 59 tests excluded from table 1, 23 were in California and cover all years from 1938 to 1944 except 1942. None of these involved a susceptible stem-rust reaction in Baart 38, nor an extremely high incidence of stem rust in Baart. Nevertheless, the average of these California tests, in which there was 31 per cent of rust-damaged tissue in Baart and only 1 per cent in Baart 38, produced an average difference of 1.3 pounds in test weight and 5 bushels in yield, as shown in table 3. Although not all of the area in which Baart is grown in California is subject to as frequent or as severe an incidence of rust as was experienced at Davis from 1938 to 1944, some areas are far more vulnerable. Also the state-wide severe losses of 1940 and 1941 must not be overlooked. It is safe to assume an average annual yield advantage of 10 per cent for one fourth of the 160,000 acres of Baart 38 that is now being grown. Since rust is more common on fertile lands, the average yield of Baart is about 40 bushels per acre. On this basis, the release of Baart 38 has added 160,000 bushels annually to California's wheat production. This is accomplished without any change in wheat acreage or change in the basic varietal type. Furthermore, Baart 38 tests on the average 1.3 pounds more per bushel where rust occurs and also tests slightly more than Baart in other sections—an advantage to the miller. Similar annual yield increases and production security followed the release of White Federation 38, which also is grown on approximately 160,000 acres in California.

TABLE 3

DIFFERENTIAL ANNUAL DEVELOPMENT OF STEM RUST ON BAART AND ON BAART 38 AT DAVIS, WITH RELATED YIELD AND TEST-WEIGHT DATA

Test year*	Stem rust		Test weight		Acre yield	
	Baart *per cent*	Baart 38 *per cent*	Baart *pounds*	Baart 38 *pounds*	Baart *bushels*	Baart 38 *bushels*
1938	5	0	60.7	61.7	46.0	49.2
1939	10	tr	64.5	64.2	54.2	52.6
1940	30	0	62.2	63.1	48.8	53.7
1941*a*†	20	0	61.4	62.7	39.0	36.1
1941*b*†	60	tr	58.4	61.4	29.1	36.4
1943	60	tr	59.2	63.0	33.7	46.0
1944	30	3	62.1	61.7	47.5	58.5
Mean	31	1	61.2	62.5	42.6	47.5

* No stem rust developed in the 1942 tests.

† 1941*a*, seeded November 26, 1940; 1941*b*, seeded January 31, 1941.

For the region as a whole, the occurrence of rust was less frequent than in California. In a few instances rust races appeared to which Baart 38 is nearly as susceptible as is Baart. Nevertheless, an average of all the 59 tests in which rust injury was recorded showed a bushel weight difference of 1.3 pounds, and a yield difference of 3.5 bushels in favor of Baart 38.

Eight other backcross-derived varieties have been compared with their commercial prototypes in 13 or more tests in which their resistance to rust, bunt, or Hessian-fly injury was of no advantage. These data are summarized in table 4. Four of these varieties were grown in additional states, but Sonora 37, Big Club 37, Poso 42, and Big Club 43 were tested only in California.

TABLE 4

COMPARISON OF YIELDS AND THEIR UNDERLYING BINOMIAL DISTRIBUTIONS FOR BASIC AND BACKCROSS-IMPROVED STOCKS OF WHEAT VARIETIES IN WESTERN REGIONAL TESTS*

Varieties compared	Comparative data			Analysis of differences			
	Number of comparisons	Mean yields per acre		Yield differences	Number of times superior in yield		Number of identical yields
		Prototype variety	Improved variety		Prototype variety	Improved variety	
		bushels	*bushels*	*bushels*			
Baart vs. Baart 38	329	39.59	39.45	−0.14	167	151	11
White Federation vs. White Federation 38	217	38.27	38.58	+0.31	105	105	7
Onas vs. Onas 41	136	52.59	52.72	+0.13	69	64	3
Federation vs. Federation 41	137	50.70	50.41	− .29	68	64	5
Pacific Bluestem vs. Pacific Bluestem 37	85	34.88	36.15	+1.27	38	43	4
Big Club vs. Big Club 37	44	38.54	37.30	−1 24	25	19	0
Sonora vs. Sonora 37	14	36.18	36.00	−0.18	7	5	2
Poso vs. Poso 42	16	33.66	32.64	−1.02	9	3	4
Big Club vs. Big Club 43	13	33.86	33.63	−0.23	6	7	0

* Exclusive of environments known to be favorable to the improved variety.

In no case has a significant mean yield difference between a commercial variety and its backcross-derived successor been established. Nonsignificant differences are either positive or negative, according to the variety comparison involved. Hence, it must be concluded that all pairs are substantially alike in their aggregate yield performance over a wide diversity of environments. The data were compiled by individual stations but regional averages only are reported here. At only one station—Aberdeen, Idaho—does the difference in yield appear to be significant. Federation, with an average yield of 82 bushels, exceeded Federation 41 by 4.4 bushels, being superior in 11 of the 13 comparisons. With this exception none of the differences in agronomic data appear to be significant.

Quality. Each season certain varieties under test in the western region are subjected to milling, baking, and other physical and chemical tests (Fifield, *et al.*, 1941). The averages of all such tests with experiment-station samples of the four principal variety pairs grown during the years 1938 to 1943 are shown in table 5. Some modifications in analytical procedure and baking formulas have occurred during the experiments, but for purposes of gross evaluation these have been disregarded in computing averages.

TABLE 5

QUALITY CHARACTERISTICS OF FOUR BACKCROSS-DERIVED VARIETIES AND THEIR COMPARABLY GROWN PROTOTYPES

(Based on averages from a number of locations in the western states during two or more years from 1939 to 1943)

Variety	Station-years	Crop years	Acre yields	Analysis of wheat								Analysis of flour	
				U. S. grade	Hard kernels	Test weight *	Flour yield†	Protein‡	Carotenoid	Pearling index	Dough-ball time	Protein‡	Ash‡
	number	*number*	*bu.*		*per cent*	*lbs.*	*per cent*	*per cent*	*p.p.m.*	*per cent*	*min.*	*per cent*	*per cent*
Baart	42	6	48.8	1 H.Wh.	90.7	62.1	70.0	11.7	2.23	41.4	54	10.2	0.52
Baart 38	42	6	48.2	1 H.Wh.	90.5	62.5	70.6	11.9	2.22	40.8	60	10.5	0.54
White Federation	35	5	49.2	1 H.Wh.	86.8	61.2	71.3	11.1	2.48	32.1	150	10.2	0.51
White Federation 38	35	5	49.8	1 H.Wh.	89.1	60.8	71.3	11.4	2.37	32.9	159	10.5	0.50
Onas	14	2	54.6	1 S.Wh.	64.0	60.9	70.6	9.4	3.03	29.4	105	8.0	0.50
Onas 41	14	2	55.5	1 S.Wh.	62.5	60.9	71.3	9.5	3.03	30.2	111	8.3	0.51
Federation	14	2	52.5	1 S.Wh.	62.5	60.2	71.0	9.7	3.25	27.0	63	8.3	0.49
Federation 41	14	2	52.5	1 S.Wh.	70.5	60.6	71.7	9.9	3.37	27.8	68	8.5	0.45

Variety	Yellow loaf cake				Cooky test			Bread test								
	Volume	Grain and texture	Crumb color	Symmetry	Factor W/T§	Color	Top grain	Absorption	Loaf volume		Grain		Texture		Crumb color	
									Formula 1¶	Formula 2¶	Formula 1¶	Formula 2¶	Formula 1¶	Formula 2¶	Formula 1¶	Formula 2¶
	cc	*score*	*score*		*ratio*			*per cent*	*cc*	*cc*	*score*	*score*	*score*	*score*	*score*	*score*
Baart	950	103	101	G	4.93	B	G	53.7	575	590	70	74	77	81	71	76
Baart 38	978	105	102	G	5.03	B	G	54.0	545	554	63	68	69	75	66	69
White Federation	905	86	96	F	4.51	LB	P	60.8	634	632	80	83	93	91	77	78
White Federation 38	893	81	93	F	4.48	LB	P	61.5	620	679	79	88	91	94	77	83
Onas	965	105	98	G+	5.99	B−	G+	54.0	481	535	63	70	55	68	65	75
Onas 41	928	105	98	G	5.65	B	G+	54.0	465	520	55	70	61	68	63	68
Federation	942	105	95	G	5.71	B	VG	54.0	478	521	58	63	61	68	53	65
Federation 41	983	103	95	G+	5.37	B−	VG	54.0	495	530	58	68	61	68	60	73

* Dockage free. † Moisture free. ‡ 13.5 per cent moisture. § Width divided by thickness.

¶ Formula 1 is the commercial formula; formula 2 is the same with bromate added in all years but 1942 and 1943, when ¼ per cent malted wheat flour was added.

The greatest number of tests were with Baart and Baart 38, and the greatest differences also were found between these paired varieties. Perhaps the most significant difference between Baart and Baart 38 is in their bread-making properties. Baart 38 has rather consistently produced lower loaf volumes, and grain, texture, and crumb-color values. These differences are small, but nevertheless appear to be significant, especially when considered in relation to the slightly higher protein content in Baart 38. Thus while it is not improbable that some other formula for making bread might make as good bread from Baart 38 as any shown here for Baart, Baart 38 apparently must be admitted to have slightly different quality attributes from Baart. This is further evidenced by the slight superiority of yellow loaf cake and of cookies baked from Baart 38. Other quality differences between Baart and Baart 38 shown in table 5 probably are not real. Many of the tests suggest close similarity rather than any difference between Baart and Baart 38.

Quality averages for three other backcross-derived varieties and their prototypes also are reported in table 5. For these, and for three other derivatives tested for only 1 year—Pacific Bluestem 37, Big Club 37, and Sonora 37—the data suggest general similarity in quality to their comparably grown prototypes. If some of the small differences are real, further testing will be necessary to establish the reality. In any case a fairly close approximation of the physical and chemical, as well as the baking responses of the prototype varieties obviously has been obtained by the backcross method of breeding as practiced in California.

DISCUSSION

From the data presented, critics of the backcross method can readily point to differences, and supporters even more readily to similarities. As the person who has been privileged to observe and study the performance of these varieties most extensively and intensively, the writer has sought to give this new breeding method a strictly objective evaluation.

In paired plantings, even a skilled agronomist usually cannot detect any morphological differences between the backcross-derived varieties and their prototypes, much less distinguish them. In rare instances, both can be done, usually by the manifestation of greater variability within the backcross-derived population. In this connection, it is important to recall that all of these varieties resulted from the making of a rather large number of crosses in each backcross generation and finally merging many F_3 or F_4 lines. It is this bulking of lines which probably accounts for the aggregate performance being so like that of the commercial variety under all environments, and yet makes possible minor aggregate differences in yield, test weight, plant height, and quality. That these or any other undetermined aggregate differences are more than minor differences certainly does not seem likely from the data at hand. Thus, there is justification for use of the common name, and general substitution of the backcross-improved stock wherever its imparted resistance is useful.

The variability occurring in the improved variety is both useful and dangerous. If a nonrecurrent parent in the pedigree of a backcross-derived variety possesses some desirable trait that has not been eliminated through conscious selection, it can probably be recovered by extensive selection. The recent recovery of selections of Baart 38 with purple straw, light red seeds, and stripe-rust resistance, are indicative of the possibilities, as are the selections from Federation 41 that are superior in winter hardiness or mildew resistance.

The diversity occurring is desirable when a variety is being improved by a succession of backcrosses with different varieties. Independent advanced breeding programs underway in California anticipate production of: (1) awnless; (2) short-strawed; (3) early-maturing; and (4) bronze-glumed forms of Baart that are resistant to both smut and stem rust. Two further backcrosses to Baart recently have been made partly to utilize the maximum level of stem-rust resistance extant in Baart 38, but more especially to minimize the genetic diversity from Baart. This stock, to be known as Baart 46, may never be grown commercially but it will be used as the recurrent parent instead of Baart in concluding backcrosses for the production of the new types mentioned above and for other future crossing. Thus, while only five or six backcrosses were necessary to achieve the high similarities reported herein, two or more additional backcrosses seem desirable when more than three improvements are to be consolidated into one improved variety.

SUMMARY

The average yields of nine backcross-derived varieties grown in paired comparisons with their commercial prototypes in tests in nine western states failed to show any general significant differences in yield between the paired strains when disease and insect injury were absent or the same for both strains.

However, significant differences in yield were evident at two stations. At Tetonia, Idaho, there was an 11 per cent yield advantage for Baart as compared with Baart 38 and at Aberdeen, Idaho, Federation showed a 5 per cent advantage over Federation 41.

From an average of more than 60 tests, Baart 38 excelled in test weight by 0.33 pound and was 0.36 inch shorter than Baart. Though these differences have a high mathematical probability of being real, they represent differences of only 0.6 and 0.9 per cent, respectively. Baart 38 was inferior to Baart in bread-making quality.

All of the data emphasize the high degree of similarity between varieties and their progenies produced from five or more backcrosses. Substitution for existing commercial varieties of any of the improved varieties herein considered, or of any subsequently produced by similar successful breeding methods, seems desirable wherever the effected improvements will operate to minimize production losses. Except for the specified improvements, the general performance of the improved varieties has been indistinguishable from those of the progenitor varieties.

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NEWLY DISCOVERED LEAFHOPPER VECTORS OF CALIFORNIA ASTER-YELLOWS VIRUS[1]

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INTRODUCTION

SOME YEARS AGO three species of leafhoppers and a biological race of one of them were reported to transmit the California aster-yellows virus (Severin, 1929, 1934, 1940).[3] Recently eight additional leafhopper species were added to the list of vectors of this virus (Severin, 1945, 1946, 1947).

The present paper deals with five newly discovered leafhopper vectors of this virus. In a companion paper DeLong and Severin (1947) discuss the characters, distribution, and food plants of these species.

An investigation was undertaken on the efficiency of the vectors in transmitting the virus by single males and females to healthy celery and asters. Multiple-lot tests were performed with several species. With three species, studies were made of the retention of the virus in single adults. Natural infectivity tests were made with two species. With some species, attempts were made to transmit the viruses of curly top to sugar beets and Pierce's disease of grapevines to grapevine cuttings or seedlings and the identical virus from alfalfa dwarf to healthy alfalfa.

The technique and equipment have been described in previous papers (Severin, 1930, 1931, 1945, 1946, 1947).

CLOANTHANUS IRRORATUS (VAN DUZEE)

(Plate 1, *A*, *B*)

Transmission of Virus to Celery and Asters. Fifty recently molted males and 50 females of *Cloanthanus irroratus* (Van Duzee), which had completed the nymphal stage on diseased celery, were kept singly on 100 celery plants until symptoms of the disease developed or during adult life if no symptoms appeared. Table 1 shows that only 1 of 50 males caused an infection, and none of the 50 females.

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[1] Received for publication August 13, 1945.

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[3] See "Literature Cited" for citations, referred to in the text by author and date.

Lots of 5, 10, 20, and 40 adults were transferred from infected to healthy celery plants and when symptoms of the disease developed, each lot was changed to a healthy aster. If no symptoms appeared on celery at the end of approximately 2 months, then the surviving adults in each lot were transferred and kept on a healthy aster until the last leafhopper died. There was a progressive increase in the percentages of infections of celery with lots of 5, 10, 20, and 40 leafhoppers, as shown in table 1, indicating that the number of leafhoppers plays an important role in the transmission of the virus. There was, however, no progressive increase in the percentages of infection of asters.

TABLE 1

TRANSMISSION OF VIRUS TO CELERY AND ASTERS, BY VARYING NUMBERS OF ADULT *Cloanthanus irroratus*

Number of lots	Number and sex of adults in each lot	Celery (first set of plants)			Asters (second set of plants)		
		Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected
50	1 male	50	1	2	..	..	..
50	1 female	50	0	0	..	..	..
60	5 males	60	6	10	60	1	2
63	10 males	63	8	13	63	0	0
53	20 males	53	14	26	53	0	0
77	40 males	77	41	53	77	8	10

TABLE 2

TRANSMISSION OF VIRUS TO ASTERS BY VARYING NUMBERS OF ADULT *Cloanthanus irroratus*

Number of lots	Number and sex of adults in each lot	Asters inoculated	Asters infected	Per cent infected
50	1 male	50	1	2
50	1 female	50	0	0
10	20 males	10	0	0
43	40 males	43	8	19

Since a mortality of the leafhoppers occurred on healthy celery in the previous experiment, another test was made with lots of varying numbers of males or females transferred from diseased celery to healthy asters. Table 2 shows that only 1 aster was infected by 50 males and 50 females tested singly on asters. Lots of 20 males caused no infections. Lots of 40 males infected 19 per cent of the asters as compared with 10 per cent in the previous experiment (table 1).

Retention of Virus by Single Adults. The retention of the virus was determined with the only leafhopper—a male—that produced an infection of celery in the single-insect tests (table 1). The first celery plant showed symptoms at the end of 19 days. The leafhopper was then transferred daily to successive healthy celery plants during adult life; it transmitted the virus to another celery plant 15 days after the first infection. The incubation period of the disease of the first infection cannot be included in the retention of the virus, since the leafhopper had an opportunity to acquire additional virus after the first celery plant became infected. The longevity of the male was 76 days.

Attempts to Transmit Viruses of Curly Top and Pierce's Disease of Grapevine. Seventeen lots of 20 male *Cloanthanus irroratus*, after feeding on curly-top beets from 1 to 2 weeks, failed to transmit the virus to 17 healthy beets. Fifteen lots of 40 noninfective *C. irroratus* failed to transmit the virus of Pierce's disease of grapevines to healthy grape seedlings and to plants of California Common or Chilean alfalfa, *Medicago sativa;* or from alfalfa dwarf to healthy grape and alfalfa plants.

CLOANTHANUS DUBIUS (VAN DUZEE)

(Plate 1, *C*, *D*)

Transmission of Virus to Celery. The efficiency of *Cloanthanus dubius* (Van Duzee) in transmitting the virus was determined with 50 males and 50 females, each kept singly on a healthy celery plant. If symptoms of the disease developed, each surviving leafhopper was used in determining the retention of the virus, and if no symptoms appeared, the adults were kept on each healthy celery plant during adult life. Eleven males and 2 females, or 13 per cent, caused infections.

Tests were made on the transmission of the virus by lots of 20 and 40 males, each transferred weekly to 6 successive healthy celery plants, and by lots of the same number of males transferred at intervals of 2 weeks. As table 3 shows, the total percentage of infection in weekly inoculations was 23 both for lots of 20 and for lots of 40 adults, as compared with 27 and 47 per cent with lots of 20 and 40 males at 2-week intervals.

TABLE 3

TRANSMISSION OF VIRUS TO SUCCESSIVE CELERY PLANTS BY VARYING NUMBERS OF MALE *Cloanthanus dubius*

Period of inoculations and number of adults in each lot	Plants infected in each set*						Total		
	First set	Second set	Third set	Fourth set	Fifth set	Sixth set	Plants inoculated	Plants infected	Per cent infected
Weekly inoculations:									
20	1	1	1	2	2	0	30	7	23
40	1	1	1	1	1	2	30	7	23
Inoculations every 2 weeks:									
20	1	3	2	0	0	1	30	8	27
40	4	4	4	1	1	0	30	14	47

* Five plants inoculated in each set.

Attempts to Transmit Virus to Asters. Fifty males and 50 females, each kept singly on healthy asters, failed to cause infections. The males lived from 1 to 12 days, an average of 3.3 days, and the females from 1 to 7 days, an average of 2.4 days, on healthy asters.

One lot of 40 males was caged on a healthy aster and after 2 weeks the surviving adults, 7 in number, were transferred to a second aster, but no infection occurred.

Retention of Virus by Single Adults. The retention of the virus was ascertained with adults previously used in determining the efficiency of the vector in transmitting virus by single insects. After the first infection the leafhoppers were transferred daily to successive healthy celery plants during adult life.

TABLE 4

RETENTION OF VIRUS BY SINGLE ADULTS OF *Cloanthanus dubius* WITH CELERY AS THE HOST PLANT

Insect no. and sex	Days on first plant before symptoms developed	Plants inoculated after initial infection	Plants infected after initial infection	Per cent infected after initial infection	Days after initial infection on which successive infections occurred	Longevity of adults, days
No. 1, male	36	28	4	14	17, 22, 27, 28	64
No. 2, male	22	55	3	5	23, 24, 29	77
No. 3, male	28	135	2	2	1, 2	163
No. 4, male	46	5	1	20	2	51

Table 4 shows that the virus was retained from 1 to 29 days. As with *Cloanthanus irroratus*, the incubation period of the disease of the initial infection is not included in the retention of the virus.

Attempt to Transmit Curly-Top Virus. Extensive tests were made to determine whether this species of leafhopper was a vector of the curly-top virus. All attempts to transmit the curly-top virus to healthy sugar-beet seedlings were negative.

EUSCELIS MACULIPENIS DeLONG AND DAVIDSON

(Plate 1, *E*, *F*)

Transmission of Virus to Celery. The efficiency of *Euscelis maculipenis* DeLong and Davidson in transmitting the virus was determined with recently molted males and females, reared during the nymphal stages on diseased celery, and then each adult was transferred singly to a healthy celery or aster plant. Single males and females infected 78 and 76 per cent of the celery plants, respectively, but none of the 100 asters inoculated, as shown in table 5.

When symptoms developed on celery, some of the insects were transferred singly to a second celery plant. These leafhoppers had an opportunity to acquire the virus again from the first infected celery. Eighteen of 21 males infected 18 of 21 plants, or 86 per cent. Seventeen females each infected a celery plant, but when transferred singly to healthy asters failed to infect any.

The longevity of the males on healthy asters ranged from 1 to 35 days, with an average of 3 days; females 1 to 24 days, with an average of 3 days.

TABLE 5

TRANSMISSION OF VIRUS BY THREE VECTORS TESTED SINGLY TO TWO HOST PLANTS

Vector	Tests on celery			Tests on aster		
	Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected
Euscelis maculipenis:						
Males	50	39	78.0	50	0	0
Females	50	38	76.0	50	0	0
Short-winged aster leafhopper, *Macrosteles divisus:*						
Males	0	..	....	125	82	66
Females	100	69	69.0	25	22	88
Long-winged aster leafhopper, *Macrosteles divisus:*						
Males	0	..	....	125	80	64
Females	100	9	9.0	25	13	52

TABLE 6

RETENTION OF VIRUS WITH SINGLE ADULT *Euscelis maculipenis* WITH CELERY AS THE HOST PLANT

Sex and number of vector	Days on first plant before symptoms developed	Plants inoculated after first infection	Plants infected after first infection	Per cent infected after first infection	Retention of virus, days	Longevity of adults, days
Males:						
No. 1	17	60	19	32	59	77
No. 2	41	50	11	22	50	91
No. 3	42	50	6	12	50	92
No. 4	36	32	11	34	31	68
No. 5	35	30	8	27	29	65
No. 6	31	29	8	28	29	60
No. 7	51	30	8	27	20	81
No. 8	14	14	10	71	13	28
No. 9	17	98	5	5	5	115
No. 10	17	108	3	3	5	125
No. 11	15	2	2	100	2	17
No. 12	36	5	1	20	4	41
Females:						
No. 1	24	4	2	50	13	28
No. 2	19	10	6	60	9	29
No. 3	65	8	5	63	7	73
No. 4	45	10	6	60	7	55
No. 5	65	8	5	63	7	73
No. 6	21	7	3	43	7	28
No. 7	32	7	1	14	7	39
No. 8	24	7	2	29	5	31
No. 9	15	5	2	40	5	20
No. 10	35	4	1	25	4	39
No. 11	47	4	2	50	3	51
No. 12	61	3	0	0	0	64

Retention of Virus by Single Adults. The retention of the virus was determined with single males and females that had transmitted the virus in tests of vector efficiency. Each insect was provided with a healthy celery plant daily during adult life. The results appear in table 6. As this table shows, the males retained the virus from 2 to 59 days, and the females from 3 to 13 days. The period before symptoms developed on the first celery plant is not included in virus retention, since the adult was able to recover the virus again. One male produced only 1 infection.

Natural Infectivity. Plantain, or ribgrass, *Plantago major*, growing in an alfalfa field near Milpitas and along roadsides, was found commonly infected with aster yellows. Nymphs and adults were often collected in this alfalfa field in the spring, but during the autumn the adults were rarely taken. Two adults captured in this alfalfa field on August 23, 1945, did not transmit the virus to celery.

Sweepings were made on common dandelion, *Taraxacum vulgare*, growing in South San Francisco, on August 20, 1945, and 3 nymphs and 30 adults were taken in half an hour. Some of the dandelion plants were infected with aster yellows, but the virus was not transmitted to celery.

Attempts to Transmit Viruses of Curly Top and Pierce's Disease of Grapevines. Ten lots of 5 adults, after feeding on curly-top beets for a period of 4 days, failed to transmit the virus to 10 healthy sugar-beet seedlings. The longevity of the adults ranged from 18 to 69 days.

No success was achieved in attempts to transmit the virus of Pierce's disease of grapevines from diseased vines to healthy wild-grape seedlings or from alfalfa plants infected with dwarf to healthy grape seedlings with this leafhopper.

MACROSTELES DIVISUS (UHLER)

Further Tests of Efficiency of Virus Transmission. In working with a large number of newly discovered leafhopper vectors of the California aster-yellows virus, it soon became evident that the ability to transmit the virus varied with different species. For the purpose of comparison, efficiency tests were conducted with the short-winged and long-winged aster leafhoppers, *Macrosteles divisus* (Uhler), which are known to be efficient vectors of the virus. The long-winged aster leafhopper is a biological race (Severin, 1940).

Short-winged and long-winged aster leafhoppers were reared on asters and on celery infected with the virus. High populations of infective short-winged aster leafhoppers were reared on diseased celery and low numbers of noninfective adults on healthy celery. Low populations of infective long-winged adults were obtained on diseased celery, but the nymphs died after the first molt on healthy celery.

Transmission of Virus to Celery and Asters. The efficiency of both vectors in transmitting the virus was determined with single leafhoppers. Twenty-five to 125 males and females that had completed the nymphal stages on diseased celery or asters were kept singly on healthy celery or asters until symptoms developed, or during adult life if no symptoms appeared. The results obtained are given in table 5.

As shown in table 5, short-winged aster leafhoppers tested singly infected 69 per cent of the celery plants, and 66 to 88 per cent of the asters, or an average of 69 per cent. Long-winged aster leafhoppers infected 9 per cent of the celery and 52 to 64 per cent of the asters, or an average of 62 per cent.

The longevities of 4 long-winged males which caused infections were 1, 10, 31, and 42 days, or an average of 26 days, and of 5 females 4, 6, 22, 28, and 42 days, or an average of 20 days. The longevity of 46 males which failed to cause infection ranged from 1 to 40 days, with an average of 6 days, and of 45 females from 2 to 38 days, with an average of 14 days.

Natural Infectivity. A comparison was also made of the natural infectivity of short-winged aster leafhoppers with *Euscelis maculipenis* collected on the same dates in the same habitats. Fifteen short-winged aster leafhoppers captured in an alfalfa field near Milpitas transmitted the virus to asters, but *E. maculipenis* failed to transmit it to celery. Eleven short-winged aster leafhoppers taken on common dandelion growing in South San Francisco transmitted the virus to asters, but 3 nymphs and 30 adults of *E. maculipenis* failed to infect celery.

FIEBERIELLA FLORII (STÅL)

(Plate 1, *G*, *H*)

Adult *Fieberiella florii* (Stål) collected in the field were kept on infected celery for 10 days or longer to complete the latent period of the virus in the insect. Fifty males and 50 females tested singly on healthy celery plants during adult life, transmitted the virus to 9 plants, or 18 per cent, and 11 plants, or 22 per cent, respectively. Eighteen lots of 5 adults produced 8 infections, or 44 per cent.

CHLOROTETTIX SIMILIS DeLONG

(Plate 1, *I*)

Chlorotettix similis DeLong has not been reared on any food plants up to the present time. This is further discussed in a companion paper (DeLong and Severin, 1947).

Adults collected in the field were kept on infected celery plants for 10 days or longer. Eight leafhoppers were then transferred singly and kept on healthy celery plants during adult life. The rest of the leafhoppers were transferred in multiple lots to successive healthy celery plants. One of the 8 adults in single-insect tests infected 1 plant. One lot of 3 adults transmitted the virus to 1 of 2 plants. One lot of 30 adults produced 1 infection and 13 surviving adults caused another infection. Lots of 16 and 20 adults failed to transmit the virus to 5 celery plants. Of the total of 17 plants inoculated, 4 were infected, or 23 per cent. The longevities of the last adult in 2 lots were 60 and 106 days, respectively, on healthy celery.

Chlorotettix similis failed to transmit the curly-top virus. One lot of 30 adults was kept on a curly-top beet for 4 days and then transferred to a healthy beet for 2 days. The surviving adults were transferred alternately to curly top and healthy beets during adult life, but no disease resulted.

SUMMARY

With single-insect tests, the following percentages of transmission of California aster-yellows virus were obtained:

	To celery	To asters
Macrosteles divisus:		
Short-winged	69	69
Long-winged	9	62
Cloanthanus irroratus	1	1
Cloanthanus dubius	13	0
Euscelis maculipenis	77	0
Fieberiella florii	20	..
Chlorotettix similis	12	..

When from 5 to 40 insects per plant were used, percentages of transmission to celery were increased with all species tested (*Cloanthanus irroratus*, *Cloanthanus dubius*, long-winged *Macrosteles divisus*, *Fieberiella florii*, and *Chlorotettix similis*), and to aster with *Cloanthanus irroratus*. *Cloanthanus dubius* failed to transmit the virus to aster even with multiple lots. Other species were not tested in multiple lots on aster.

The virus was retained for a maximum of 15 days by *Cloanthanus irroratus*, 29 days by *C. dubius*, and 59 days by *Euscelis maculipenis*. Virus retention was not studied on other species.

Unsuccessful attempts were made to transmit the virus of Pierce's disease of grapevines to grape seedlings with two species (*Cloanthanus irroratus* and *Euscelis maculipenis*) and the virus of curly top to beets with three species (the two just listed and *C. dubius*).

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PLATE

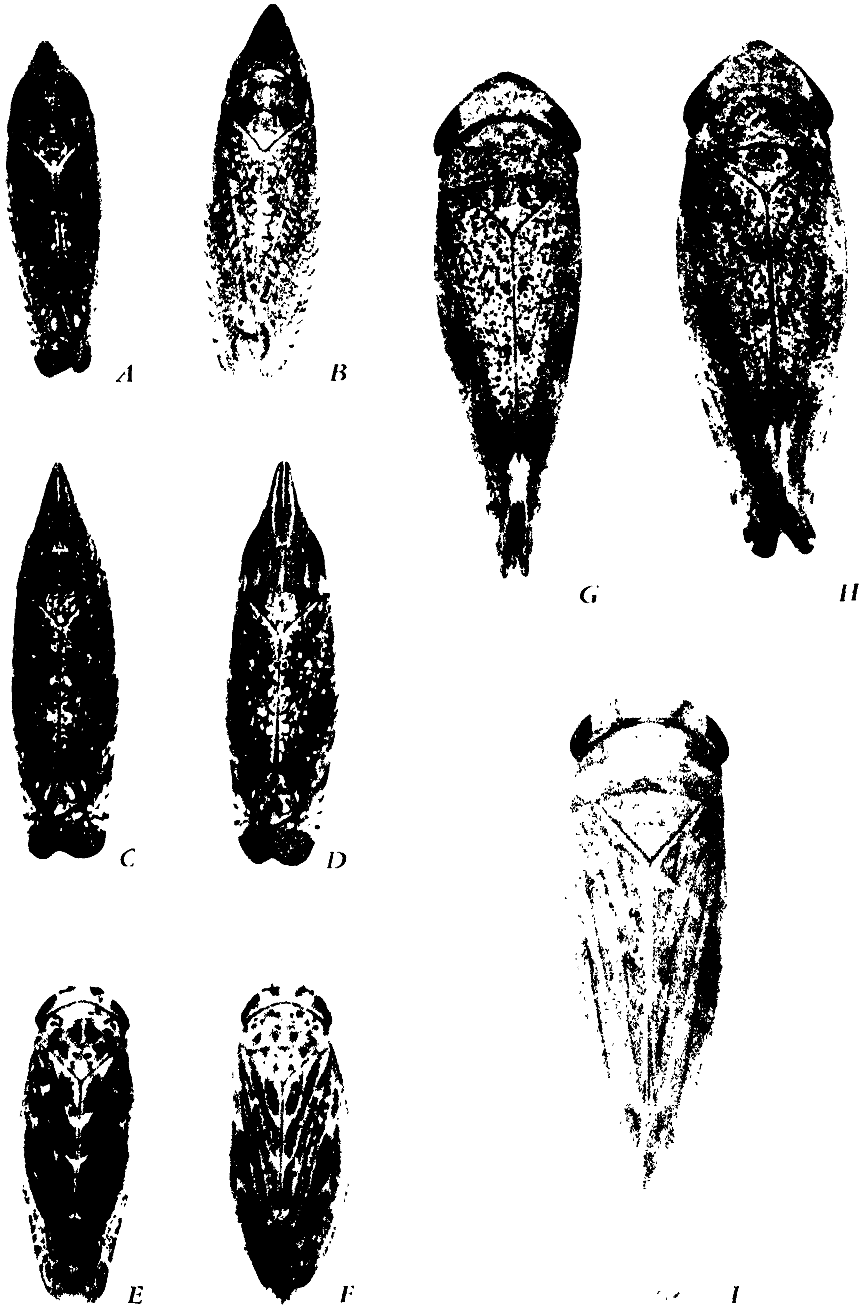

Plate 1.—*A*, Male, *B*, female *Cloanthanus irroratus* (Van Duzee); *C*, male, *D*, female *Cloanthanus dubius* (Van Duzee); *E*, male, *F*, female *Euscelis maculipenis* DeLong and Davidson; *G*, male, *H*, female *Fieberiella florii* (Stal); *I*, female *Chlorotettix similis* DeLong.

CHARACTERS, DISTRIBUTION, AND FOOD PLANTS OF NEWLY DISCOVERED VECTORS OF CALIFORNIA ASTER-YELLOWS VIRUS

DWIGHT M. DeLONG and HENRY H. P. SEVERIN

CHARACTERS, DISTRIBUTION, AND FOOD PLANTS OF NEWLY DISCOVERED VECTORS OF CALIFORNIA ASTER-YELLOWS VIRUS[1]

DWIGHT M. DeLONG[2] and HENRY H. P. SEVERIN[3]

INTRODUCTION

FIVE LEAFHOPPER SPECIES are reported in the accompanying paper (Severin, 1947)[5] as vectors of California aster-yellows virus. The insects are illustrated by colored plates in that paper. The present paper deals with the characters, distribution, and food plants of these leafhopper vectors, which belong to the genera *Cloanthanus, Euscelis, Fieberiella* and *Chlorotettix.*

CLOANTHANUS ACUTUS (SAY)

Cloanthanus acutus has not been demonstrated to be a vector of the virus, but is included here because it has been confused with other species.

The species of *Cloanthanus*, formerly placed in *Platymetopius*, have been confused in their identity for many years because an attempt has been made to determine them by color pattern alone. These species unfortunately are too similar in color to distinguish several of the common species on this basis.

After a study of the Mexican and southwestern species, DeLong (1943) used the male genital structures to distinguish them. He showed that in many cases the structures are quite different. By means of those structures he set up a neotype (1945) for one of the common eastern species, *Cloanthanus acutus* (Say, 1831) illustrating the genital structures (fig. 1) and describing several closely related species in the East. From these studies it is obvious that several common or economic species have been erroneously identified in the past, and it is apparent that typical *C. acutus* does not occur in California nor the western United States in general.

CLOANTHANUS IRRORATUS (VAN DUZEE)

Cloanthanus irroratus (Van Duzee, 1910) is a brown species with a banded vertex and an irrorate face. In length it measures from 3.5 to 4.0 mm.

The vertex (fig. 2, *A*) is strongly produced and angled, and its median length is a little more than one third greater than the basal width between the eyes.

In color the vertex is dark brown with a wedge-shaped apical spot. A series of short pale longitudinal lines form a broken transverse band before the eyes. The base is also pale. The pronotum is brown with five pale longitudinal lines.

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[1] Received for publication March 23, 1946.

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[4] The authors wish to express appreciation to R. V. Hershberger, Ohio State University, who assisted with the illustrations.

[5] See "Literature Cited" for citations, referred to in the text by author and date.

The scutellum is pale, the basal angles are orange. The elytra are rather sparsely irrorate forming several pale areas. The face is pale brown, irrorate with darker brown.

The female last ventral segment has the posterior margin produced in a broadly rounded shape.

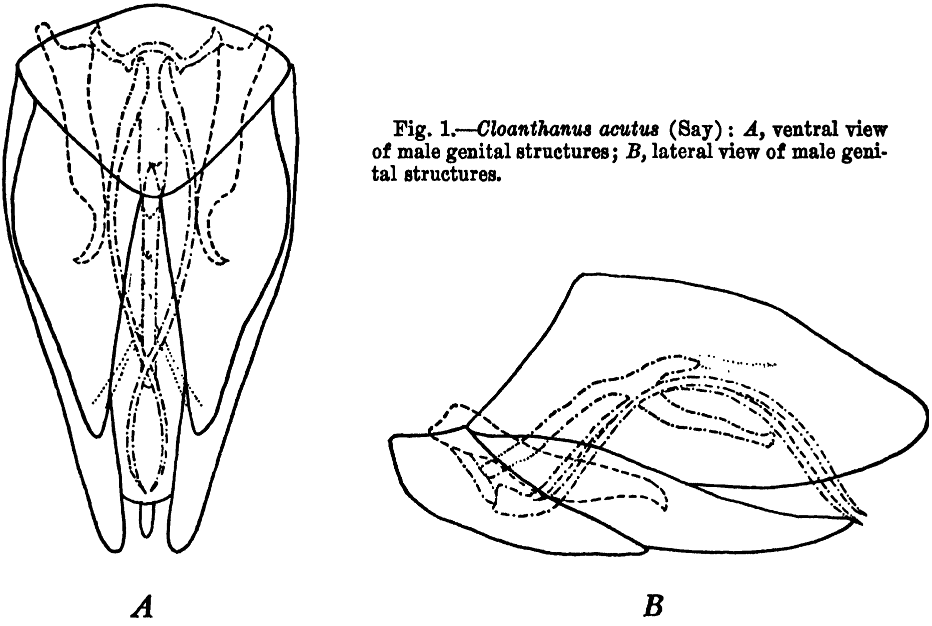

Fig. 1.—*Cloanthanus acutus* (Say): *A*, ventral view of male genital structures; *B*, lateral view of male genital structures.

The male genital plates are triangular (fig. 2, *B*). The style has a fingerlike apical process which curves outwardly (fig. 2, *C*). The aedeagus is broadly U-shaped with a basal stem. The apical portion of the stem has a pair of processes which are bent anteriorly.

The female segment is not different from that of most of the species of the genus. The male, however, is unique in the structure of the genital pieces, and the lateral view of the aedeagus will separate it at once from any of the other described North American species. A recently described Mexican species, *Cloanthanus apertus* DeLong (1943), is probably nearest in type of aedeagus. Of those species occurring in the United States *C. torridus* (Ball, 1916) is probably nearest in genital structure.

Geographic Range.—In his original description Van Duzee (1910) cited this species from Chino, Riverside, Pasadena, and San Diego, California; Phoenix, Arizona; and Tia Juana, Mexico. More recent records show its range extending to the states of Nuevo Leon and San Luis Potosi, Mexico (DeLong, 1943).

Distribution and Food Plants in California. During this study it has been collected in the following localities in California:

Riverside County: At Arlington on May 21, 1941, 2 females were obtained from an alfalfa field by N. W. Frazier.

Kern County: On December 21, 1941, at Arvin, a few adults were captured in miscellaneous sweepings by N. W. Frazier. The host plant is unknown.

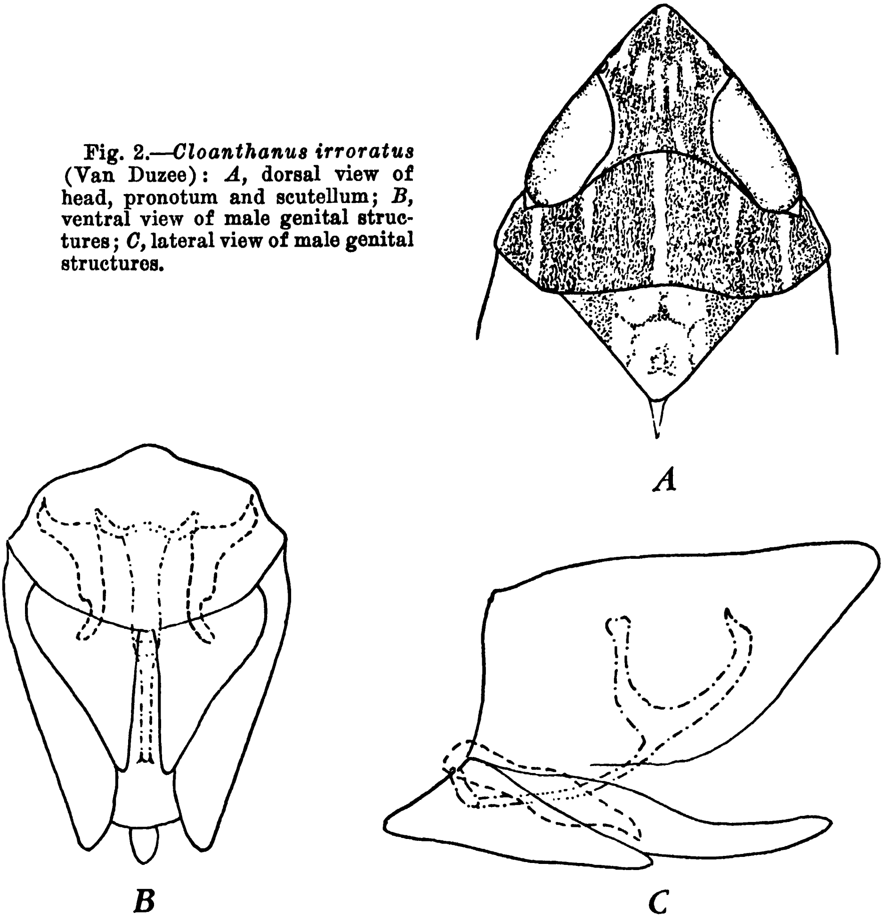

Fig. 2.—*Cloanthanus irroratus* (Van Duzee): *A*, dorsal view of head, pronotum and scutellum; *B*, ventral view of male genital structures; *C*, lateral view of male genital structures.

Madera County: At Madera on October 9, 1941, several adults were collected in an alfalfa field by H. H. P. Severin.

Sacramento County: In a locality known as Sacramento Pocket on September 12 and 18, 1941, adults were commonly taken on wild licorice, *Glycyrrhiza lepidota*, by H. H. P. Severin.

Sonoma County: Adults were caught in sweepings of mountain grape, *Vitis rupestris*, and California blackberry, *Rubus vitifolius*, at Triniti (in foothills near Cloverdale), May 8, 1940, by N. W. Frazier.

San Mateo County: On September 19, 1941, at Montara, a few adults were collected on weeds by H. H. P. Severin.

CLOANTHANUS DUBIUS (VAN DUZEE)

The leafhopper *Cloanthanus dubius* (Van Duzee) is given specific ranking in this discussion. Superficially it resembles *C. acutus* rather closely in form, color, size, and markings. Several years ago when it was named by Van Duzee (1910, 1917), he placed it as a variety of *C. acutus* and did not take space to describe it. Since more detailed study has been made of speciation and characters to designate species, it seems advisable to give it specific ranking.

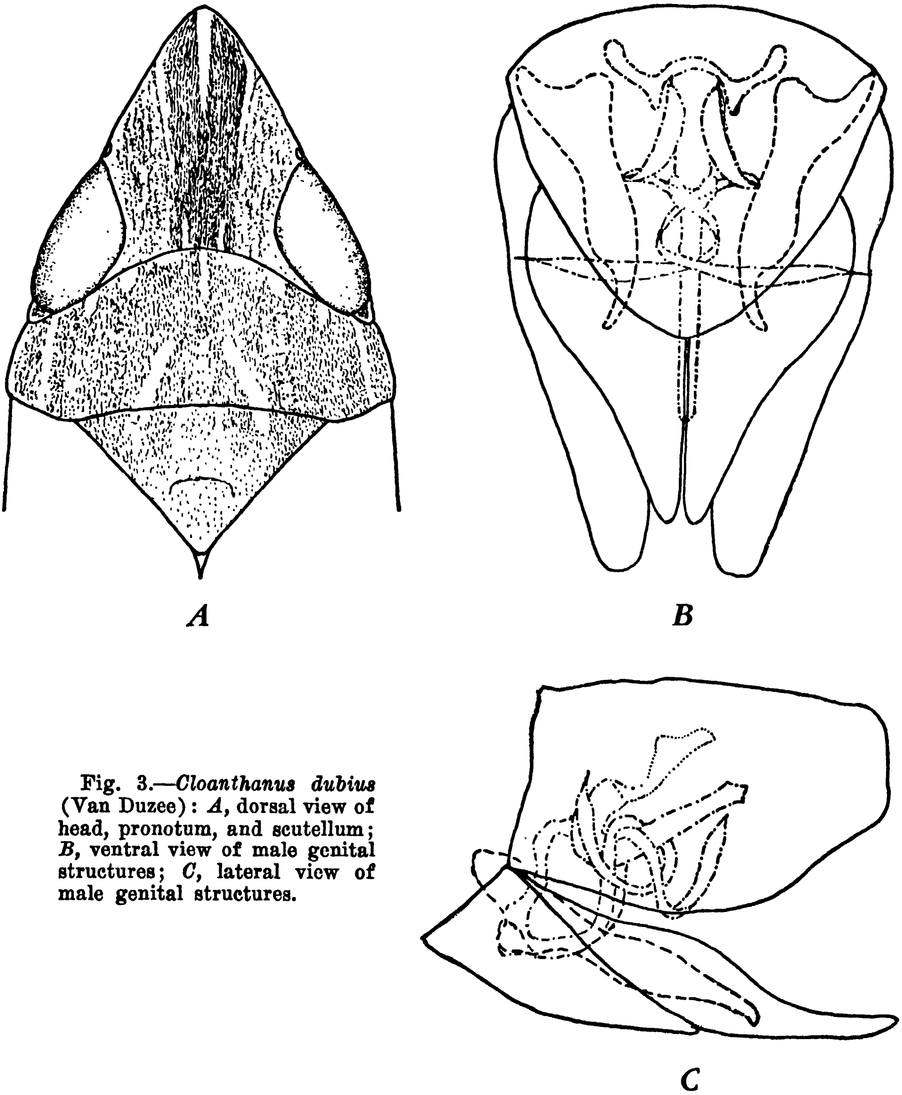

Fig. 3.—*Cloanthanus dubius* (Van Duzee): *A*, dorsal view of head, pronotum, and scutellum; *B*, ventral view of male genital structures; *C*, lateral view of male genital structures.

Cloanthanus dubius resembles *C. acutus* in form and color—dark brown with a pale face—but has distinct male genitalia (fig. 3, *B*, *C*). Its length is 5 mm.

The vertex is strongly produced and sharply angled (fig. 3, *A*). Its median length is about one and one half times as great as the basal width between the eyes.

In coloration the intensity varies from pale brown to dark brown. The male is usually darker in color than the female. The color is formed by rather closely spaced brown mottlings or rows of irrorate spots. The vertex in the male is dark brown with three slender white longitudinal stripes, one at the apex extending about one third the distance to base and one on either side about halfway from the apex to the eye, extending slightly obliquely toward base. In the female the vertex appears to have a broad dark median longitudinal band because the coloration is darker between the two lateral pale lines. The pronotum is brown with traces of five pale longitudinal lines. The scutellum has the basal angles usually darker, the central portion somewhat mottled with pale spots. The elytra have pale round areolar spots in the base or apex of cells on the corium, clavus, or in the apical or anteapical cells. The face is pale and darker just beneath margin.

The female last ventral segment has the posterior margin produced in a broadly rounded shape.

The male plates are rather short, rather broad at base, and triangular. The style is elongate, narrowed at about one third its length, and more deeply excavated on outer margin near apex to form a rather long slender fingerlike apex, which curves outward. The aedeagus is composed of a dorsal U-shaped portion and a pair of ventral processes which are long and vermiculate. The dorsal portion is rather broad, curved at base, the ventral piece of which is rather blunt at apex. The dorsal piece is much shorter and attached to a dorsal membranous portion. The ventral paired processes are long, normally curved about the ventral piece of the dorsal portion, broadened to form narrow bladelike structures on their apical fourth, and are sharply pointed at the apex.

As compared with the male genital structure of *Cloanthanus acutus*, the most striking differences are in the ventral piece of the dorsal portion and paired processes of the ventral portion. In *C. acutus* these paired processes are long and narrow, not broadened apically, and extend to the apex of the pygofer without being curved around the dorsal portion of the aedeagus.

Geographic Range. This species seems to be confined to the West Coast states and probably to California. It has been cited, apparently in error, from Quebec by Van Duzee (1908) and from Tennessee by DeLong (1916).

Distribution and Food Plants in California. Van Duzee (1914) reports this variety of leafhopper as not uncommon on the chaparral at Alpine and elsewhere in San Diego County.

N. W. Frazier collected this variety of leafhopper on pasture grasses and weeds in the following localities:

Sonoma County: Triniti, June 25, 1939; May 12, 1940; March 28, 1942; February 13, April 7, and June 4, 1943; and June 8, 1944.

Napa County: Dry Creek School, June 2, 1942; February 13, 1943.

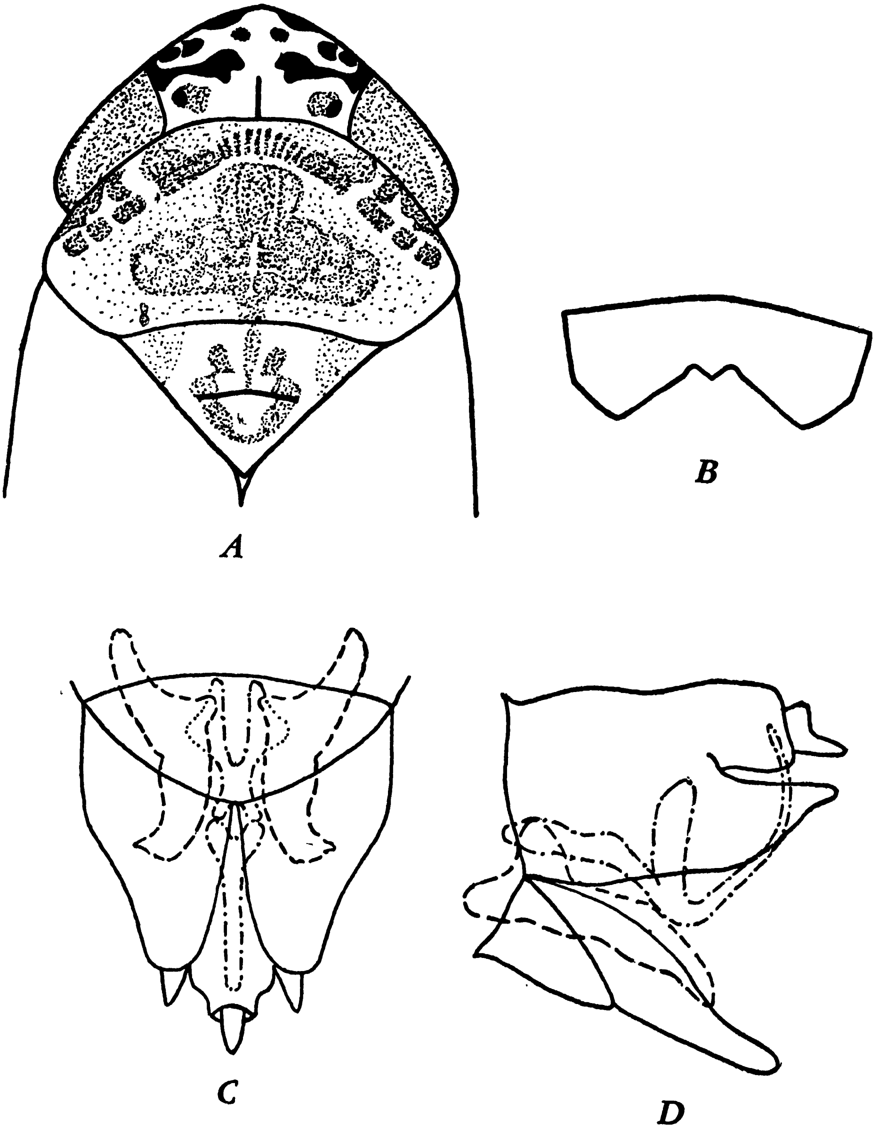

Fig. 4.—*Euscelis maculipenis* DeLong and Davidson: *A*, dorsal view of head, pronotum, and scutellum; *B*, ventral view of ninth female abdominal segment; *C*, ventral view of male genital structures; *D*, lateral view of male genital structures.

EUSCELIS MACULIPENIS DeLONG AND DAVIDSON

Euscelis maculipenis DeLong and Davidson (1934) is a rather robust species with a bluntly produced head which is marked with brown spots. It measures 5.0 to 5.5 mm in length.

The vertex (fig. 4, *A*) is bluntly produced, about one third longer in the middle than next the eyes and is more than one third wider between the eyes than the median length.

The color is somewhat variable in intensity. Usually there is a row of four black spots just above the margin and a transverse spot on either side, sometimes divided, extending from the eye above the ocellus almost to the middle of the vertex. The pronotum is marked with black spots and transverse striae. The elytra are marked with small dark brown spots, especially paralleling or between the veins. These markings are more intensified in some specimens. The apices of the elytra are infuscated.

The last ventral segment of the female (fig. 4, *B*) has a posterior margin which is broadly excavated from the rather prominent lateral angles to a median broad, slightly produced tooth, which is black-margined.

The male valve (fig. 4, *C*) is bluntly triangular and the plates are rather broad, blunt, and rounded at the apices. The styles are rather elongate with broad blunt apices and a sharp-pointed spinelike projection on the outer apical margin. The aedeagus is rather slender and U-shaped. The basal third is thickened and bent dorsally, from the base of which the more slender portion slopes caudally and dorsally to the anal tube (fig. 4, *D*).

Geographical Range. This species was originally described from specimens from Idaho. It has since been collected in Oregon and California.

Distribution and Food Plants in California. The first population of this leafhopper was collected by J. H. Freitag in a depleted, grassy, alfalfa field near Milpitas on January 8, 1943. Nymphs were commonly taken in this alfalfa field in the spring, but during the summer the adults flew to other food plants. During 1943 to 1945 adults were captured by H. H. P. Severin in the spring, summer, and autumn in vegetable fields in South San Francisco on the following economic plants: celery, *Apium graveolens* var. *dulce;* common dandelion, *Taraxacum vulgare*; endive, *Cichorium endivia;* lettuce, *Lactuca sativa;* garden, table, or red beets, *Beta vulgaris;* Swiss chard, *B. vulgaris* var. *cicla;* and New Zealand spinach, *Tetragonia expansa.* Nymphs and adults were commonly collected on the following herbs: narrow-leaf sage, *Salvia officinalis;* rosemary, *Rosmarinus officinalis;* and sweet marjoram, *Majorana hortensis.* The adults were often taken on *Artemisia vulgaris* growing along the Russian River near Geyserville and the Napa River near St. Helena and Larkmead.

FIEBERIELLA FLORII (STÅL)

Fieberiella florii (Stål) (1864) was formerly called *Philipsius atropunctatus* DeLong (1923). It is a broad, flat-headed species, the upper surface of which is almost completely covered with small round black "pepper" spots. In length it measures 7.0 to 7.2 mm.

The vertex (fig. 5, *A*) is flat and one and one half times as wide between the eyes as the median length. The anterior edge is sharp. The elytra are rather broad, opaque, and flaring at the apices. The veins are rather obscure, and the appendix is wanting.

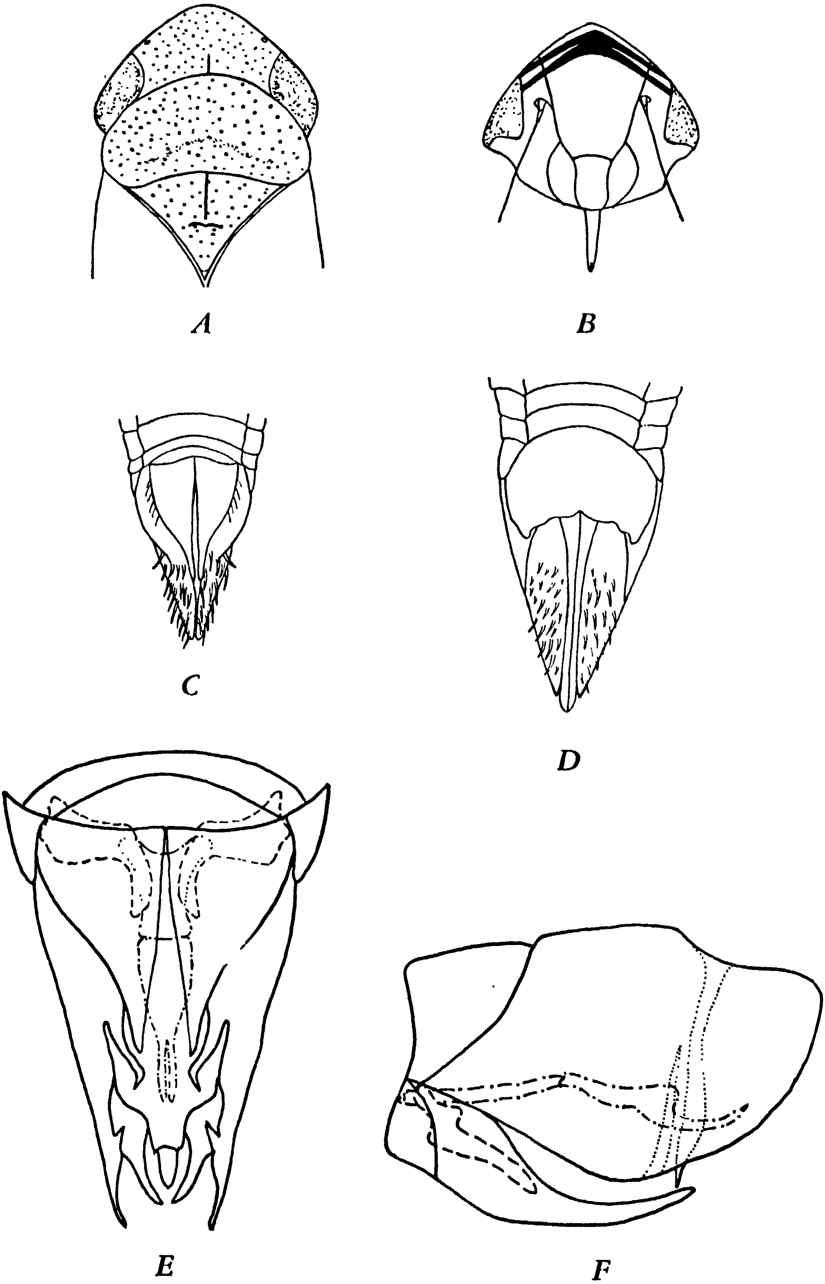

Fig. 5.—*Fieberiella florii* (Stål): *A*, dorsal view of head, pronotum, and scutellum; *B*, ventral view of head showing face; *C*, ventral view of posterior portion of male abdomen showing the valve, plates, and pygofer; *D*, ventral view of posterior portion of female abdomen showing ninth segment, pygofer, and ovipositor; *E*, ventral view of male genital structures; *F*, lateral view of male genital structures.

The vertex, pronotum, scutellum, and elytra are dull yellow, and rather densely and finely irrorate with round black spots. The veins are slightly brown and the apical veins are broadly margined with dark fuscous. The face (fig. 5, *B*) is yellowish with two heavy black bands, sometimes fused, just below the margin of the vertex. In the female the venter is white, the last ventral segment and pygofer brownish.

The female last ventral segment has rather prominent rounded lateral angles, between which the posterior margin is sinuately concave with a very shallow central notch (fig. 5, *D*).

The male plates (fig. 5, *C*) are elongate, triangular, longer than the combined width at the base and are narrowed to long acute attenuated apices. The styles are short, the apical third is rather narrow and curved outward. The aedeagus is thickened at the middle and narrowed on the apical third to form a pair of proximal fingerlike processes (fig. 5, *E*). There are two pairs of long spines on the pygofer. The longer pair are bladelike in appearance and extend from the dorsal margin to the ventral margin of the pygofer (fig. 5, *F*). The shorter pair extend dorsally from the ventral margin of the pygofer just beyond the middle of the aedeagus.

Geographical Range. This species was described from Europe and has apparently been imported into the United States upon ornamental plants. It was first found along the eastern coast and was redescribed by DeLong (1923) from Connecticut. It has since been found in Ohio, Illinois, and more recently in California. In the eastern states it is common on certain perennial ornamental plants.

Distribution and Food Plants in California. Napa County: The first population of this leafhopper was collected by J. H. Freitag on California privet (*Ligustrum ovalifolium*) growing as a hedge along the roadsides of the Beaulieu estate at Rutherford on August 9, 1944. Adults were captured by H. H. P. Severin on California privet growing on the Beaulieu estate on August 31, 1945, and on another hedge (*L. nanum* var. *compactum*) at St. Helena on the same date. On September 6 and October 4, 1945, and July 29 and August 9, 1946, adults were taken on classic myrtle (*Myrtus communis*) and on *Cotoneaster pannosa* growing on the grounds of the Napa Junior College. Nymphs were commonly collected on these host plants during April, May, and June but were rarely taken on these same host plants during the autumn. The leafhopper may overwinter in the egg stage in California, or the adults may fly to other food plants.

CHLOROTETTIX SIMILIS DeLONG

Chlorotettix similis DeLong (1919) is a pale-green, broad-headed species similar in appearance to *C. unicolor*, a common northeastern species. In length it measures from 6.5 to 7.5 mm.

The vertex (fig. 6, *A*) is broadly rounded and longer at the middle than next to the eyes. The width between the eyes is more than twice the median length.

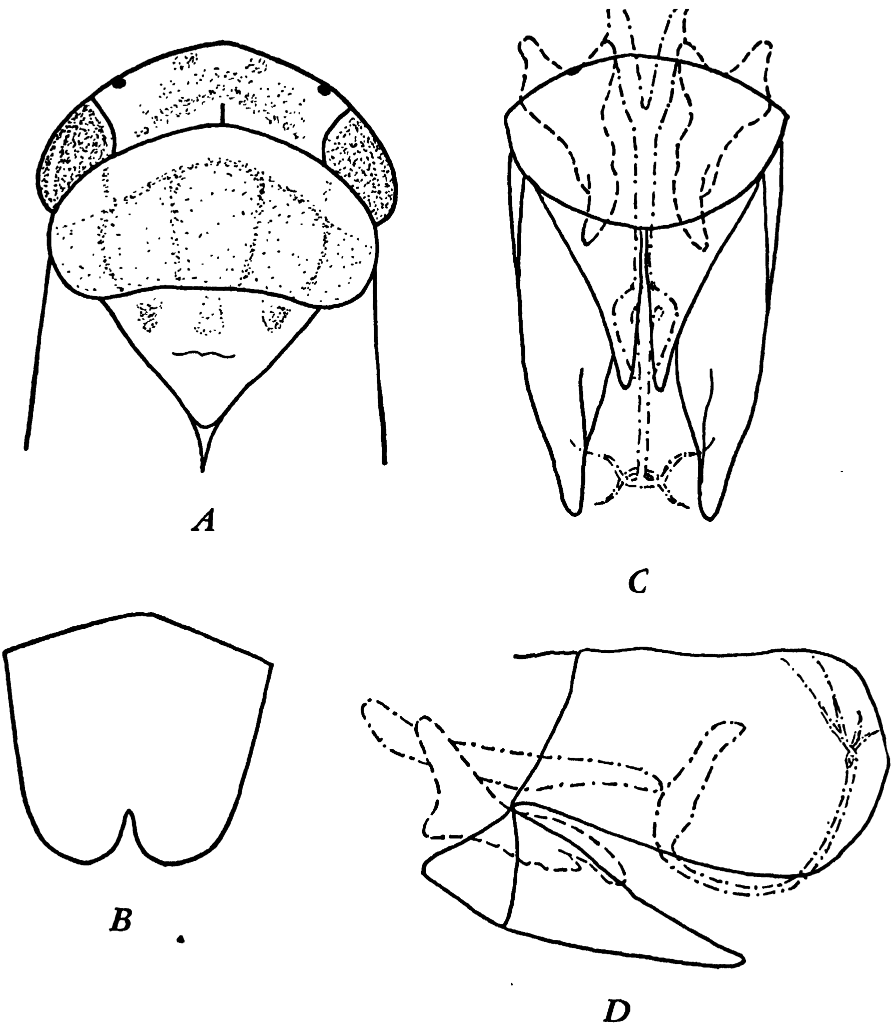

Fig. 6.—*Chlorotettix similis* DeLong: *A*, dorsal view of head, pronotum, and scutellum; *B*, ventral view of ninth segment of female; *C*, ventral view of male genital structures; *D*, lateral view of male genital structures.

In color the entire body is rather uniformly pale green without definite markings of any kind.

The female last ventral segment (fig. 6, *B*) is similar in type to that of *C. unicolor*, but is longer and more narrowed. The entire posterior margin is composed of two lobes, rather broadly rounded, which are formed by a V-shaped median notch, the sides of which are convexly rounded and after meeting, often overlap along the median line of incision. This incision extends one third of the way to the base. The whole surface of the segment is very rugose and striated, the striae running transversely on the anterior half and longitudinally on the posterior portion.

The male plates (fig. 6, *C*) are triangular, the apices are narrow and pointed. The plates are much shorter than the pygofers. The styles are elongate, narrowed to bluntly pointed apices. The aedeagus (fig. 6, *D*) is long and slender with four slender terminal processes, two of which are short and two of which are quite long. The basal portion is bent dorsally and thickened and the apical half is bent dorsally.

Geographic Range. This is apparently the common broad-headed species of *Chlorotettix* in the Pacific Northwest and in California. It was originally described from Oregon and has since been recorded for California.

Distribution and Food Plants in California. Sonoma County: At Triniti, on June 4, 8, and August 14, 1943, adults were collected in miscellaneous sweepings.

Sonoma County: On June 7, 1944, at Geyserville a few adults were collected on *Artemisia vulgaris* by H. H. P. Severin.

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LONGEVITY OF NONINFECTIVE AND INFECTIVE LEAFHOPPERS ON A PLANT NONSUSCEPTIBLE TO A VIRUS

HENRY H. P. SEVERIN

LONGEVITY OF NONINFECTIVE AND INFECTIVE LEAFHOPPERS ON A PLANT NONSUSCEPTIBLE TO A VIRUS[1]

HENRY H. P. SEVERIN[2]

INTRODUCTION

In a recent paper (Severin, 1946),[3] evidence was presented that nine species of leafhopper vectors tested, completed the nymphal stages on celery or asters infected with the California aster-yellows virus, but died when transferred to healthy celery or asters. Conceivably, this might be either (1) because the virus itself is beneficial to the vector, or (2) because the host plant is modified by virus infection to become a more favorable food and breeding plant for the vector. This paper deals with the first possibility, through a comparative study of the longevity of noninfective and infective leafhoppers on a host plant nonsusceptible to the virus. If the virus is beneficial to the vectors, it is reasonable to assume that the adult life of infective leafhoppers reared during the nymphal stages on infected host plants would be longer on a host plant nonsusceptible to the virus, than that of noninfective adults kept on the same species of nonsusceptible host plant. On the other hand, if the virus is injurious to the vectors, then the longevity of infective leafhoppers would be shorter than that of noninfective adults kept on the same species of nonsusceptible host plant.

It is not the purpose of this paper to enter into a discussion as to whether the plant has been modified by virus infection to become a more favorable host plant for the vector.

METHODS

Short-winged and long-winged aster leafhoppers were used. The latter is a biological race of the same species, *Macrosteles divisus* Uhl. The noninfective insects were reared on mildew-resistant Sacramento barley, which is nonsusceptible to the virus. Infective leafhoppers were reared on diseased China asters (*Callistephus chinensis*) and plantain, or ribgrass (*Plantago major*).

In January, soon after the last molt, lots of 50 or 100 noninfective and infective male or female leafhoppers were confined in cages enclosing Sacramento barley. Each month the leafhoppers were transferred to thrifty growing barley plants, and a record was taken of the mortality.

RESULTS

Table 1 gives the results obtained. As shown in this table, no mortality of noninfective or infective adults of either vector occurred during the first month. An examination of the mortality records in the first test of the short-

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[1] Received for publication November 4, 1946.

[2] Entomologist in the Experiment Station.

[3] See "Literature Cited" for citations, referred to in the text by author and date.

winged aster leafhoppers during the next four months shows a higher death rate of noninfective than of infective males, but the reverse is true when the data for the second test are compared.

TABLE 1

MONTHLY MORTALITY OF NONINFECTIVE AND INFECTIVE ADULT SHORT-WINGED AND LONG-WINGED *Macrosteles divisus* ON SACRAMENTO BARLEY, NONSUSCEPTIBLE TO ASTER-YELLOWS VIRUS

Sex and condition of aster leafhopper, and test no.	Adults in each lot	Cumulative mortality, per cent (Leafhoppers transferred to barley in January)					
		Feb.	Mar.	Apr.	May	June	July
Short-winged males:							
Test 1:							
Noninfective	100	0	20	34	88	97	100
Infective	100	0	19	40	92	98	100
Test 2:							
Noninfective	100	0	43	50	88	99	100
Infective	100	0	19	37	73	97	100
Long-winged males:							
Test 1:							
Noninfective	50	0	18	48	94	100	...
Infective	50	0	32	72	88	100	...
Test 2:							
Noninfective	50	0	28	54	86	98	100
Infective	50	0	30	62	88	98	100
Long-winged females:							
Noninfective	50	0	24	36	90	98	100
Infective	50	0	18	44	86	100	...

With both tests of long-winged aster leafhoppers, table 1 shows a higher mortality of infective than of noninfective adults, both male and female. The slight difference is not significant.

CONCLUSION

The results indicate that the virus itself is neither beneficial nor injurious to infective short-winged and long-winged adult aster leafhoppers, *Macrosteles divisus,* as determined by a comparison of the monthly death rate of noninfective and infective adults kept on a host plant nonsusceptible to the California aster-yellows virus.

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LOCATION OF CURLY-TOP VIRUS IN THE BEET LEAFHOPPER, EUTETTIX TENELLUS[1]

HENRY H. P. SEVERIN[2]

INTRODUCTION

THE CURLY-TOP VIRUS is sometimes transmitted by the beet leafhopper, *Eutettix tenellus* (Baker), many days after the insect has had an opportunity to acquire the virus by feeding on an infected plant. The retention period of the virus in the insect has been reported to be as long as 151 to 180 days (Freitag, 1936).[3] The question of where the virus is during this time has interested a number of investigators.

Earlier attempts to determine the location of the virus in the leafhopper were failures. In experiments at this station (Severin, 1922), not a single case of curly top was obtained by inoculating healthy beets with various internal organs from infective beet leafhoppers dissected in physiological salt, Ringer's and Locke's solutions, and in juice expressed from healthy beets. The excrement from infective beet leafhoppers inoculated into the petioles of healthy beets also failed to produce curly top. Carsner and Stahl (1924) dipped the points of steel needles into drops of fresh excreta from infective beet leafhoppers and then pricked the excrement into two beet seedlings, but no disease resulted.

Later, however, Carter (1928*a, b*) succeeded in demonstrating curly-top virus in leafhopper saliva. He transmitted the virus by previously noninfective leafhoppers which had fed on a solution on which infective insects had previously fed, the incubation period of the disease in the beets in these instances being, as a rule, prolonged. An attempt (Severin, 1931) to demonstrate its presence in feces by a similar technique was unsuccessful: previously noninfective nymphs after feeding on a feeding solution containing the feces from infective beet leafhoppers failed to transmit curly top to sugar beets. But in 1938 Bennett and Wallace reported that a small amount of virus passes through the beet leafhopper and was present in active condition in

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[3] See "Literature Cited" for citations, referred to in the text by author and date.

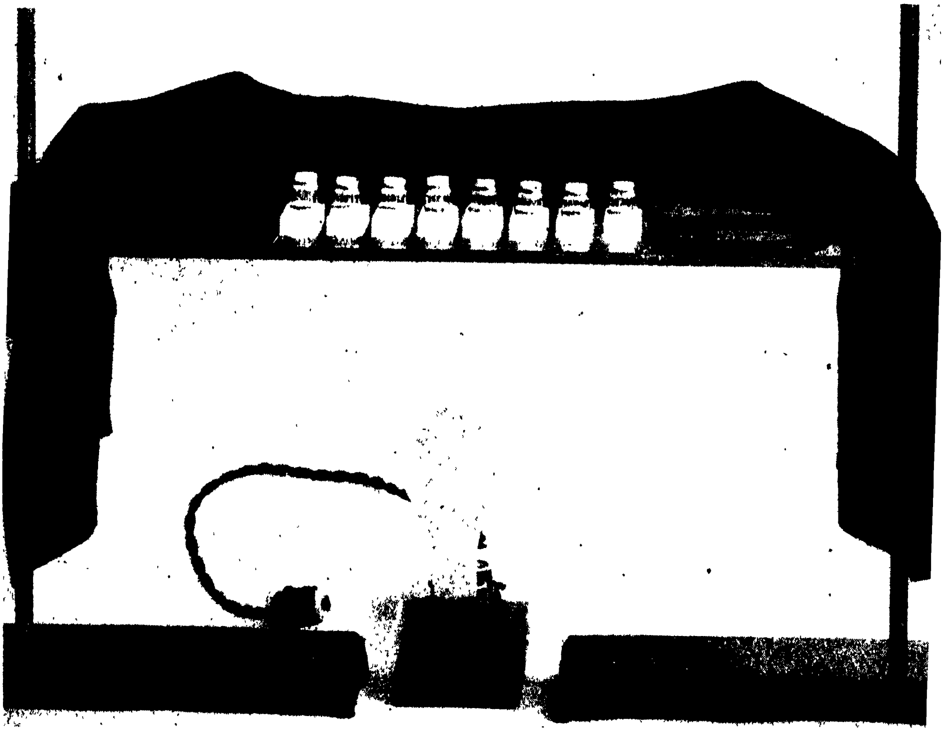

Fig. 1. Apparatus for drop method of feeding noninfective beet leafhopper nymphs on virus extract from internal organs. A drop of virus extract was placed on the "fishskin" covering the flared surface of the glass cage (see also fig. 2). The membrane with a drop of virus extract was placed over the depression of a microculture slide containing a small quantity of water. The feeding apparatus was placed on a glass plate above an electric incandescent lamp to attract the nymphs to the lighted membrane in the glass cage, and the top of the cages was covered with black sateen.

the feces. They also reported that virus was obtained from the blood, salivary glands, and alimentary canal of the beet leafhopper and concluded that the blood is the chief reservoir of the virus.

Dobroscky (1929) in her study on the aster-yellows virus in its insect vector, *Macrosteles divisus* (Uhler), suggested that "if certain portions of virus-carrying individuals were isolated and healthy insects made to feed on them, one might get an indication of the whereabouts of the virus."

This paper reports the results of further experiments to determine the location of the virus in the beet leafhopper. Attempts were made to determine whether the virus was located in the alimentary canal, salivary glands, saliva, blood, and ovaries of infective beet leafhoppers. Noninfective leafhoppers were fed on filtered solutions in which internal organs of infective insects had been crushed. Investigations were also undertaken on the filterability of the curly-top virus in the various internal organs of infective leafhoppers.

METHODS

Feeding Solution. A large number of experiments were conducted with various feeding solutions using different kinds and percentages of sugar. The solutions used were juices from the leaves, petioles, leaves and petioles, beet root, or the entire beet plant, often mixed with various amounts of different sugar solutions.

In our early work the filtrates prepared from the internal organs of infective beet leafhoppers were often incubated, sometimes for a period of 1 to 7 days. It is not necessary to enter into a discussion of these experiments, which usually gave negative results, since many other experiments have demonstrated that the curly-top virus is inactivated by prolonged incubation of aerobic filtrates.

Fig. 2. Microculture slide and glass cage used in feeding noninfective nymphs (shown on glass plate in fig. 1).

In our later work the feeding solution mostly used was as follows:

6 cc filtered healthy beet-root juice
1 cc soluble starch 2 per cent
1 cc beet sugar 2 per cent

Stahl and Carsner (1918) were the first to report a method of obtaining beet leafhoppers noninfective as to curly top. During the process of hatching from a diseased beet, the nymphs before feeding were transferred with a camel's-hair brush to a healthy beet, and the leafhoppers failed to produce the disease. Successive generations have been reared on healthy beets during a period of thirty years, and the leafhoppers remained noninfective throughout this period.

Dissections. Before dissecting out the internal organs from infective beet leafhoppers, the insects were transferred from diseased beets to an empty cage and were kept without food overnight. This was done to avoid the possible transmission of unchanged diseased beet juice in the alimentary canal. The adults were dropped into a small phial containing sterile distilled water, and the bottle was shaken so that the wings became wet and they were unable to fly. The leafhoppers were transferred to fresh distilled water in a Syracuse watch glass with the bottom covered with a mixture of paraffin and beeswax. While the insect was submerged in the distilled water the internal organs were dissected from the adults with a small triangular or spear-shaped scalpel. The internal organs were thoroughly washed with many changes of sterile, distilled water by agitating the liquid with a medicine dropper. The internal organs were transferred to the feeding solution and were crushed with a small pestle in a watch glass.

Filtration. The feeding solution was filtered through coarse and fine Berkefeld candles. Except as otherwise noted, after the internal organs were crushed in the solution each solution was again filtered through a small fine Berkefeld candle.

Feeding Leafhoppers on Virus Extracts. Carter (1927, 1928*a*) perfected methods of feeding nymphs and adult beet leafhoppers on liquids. Using this feeding apparatus in a modified form, Severin and Swezy (1928) demonstrated, from both diseased sugar beets and crushed infective beet leafhoppers, the filterability of the curly-top virus. These feeding equipments required a considerable amount of liquid and it was assumed that the dilution magnitude of the curly-top virus would play an important role in this work. Hence a feeding technique was devised in which a small quantity of solution was employed in a microculture slide as described in a previous paper (Severin and Swezy, 1928). In some of the experiments noninfective nymphs were fed on a drop of solution placed on the "fishskin" covering the flared surface of a glass cage. The membrane with the drop of liquid was placed over the depression of a microculture slide containing a small quantity of water to prevent evaporation. The feeding apparatus was placed on a glass plate above an electric incandescent lamp (figs. 1 and 2) and the top of the glass cages was covered with black sateen. The nymphs, which had been fasted for a few hours, were attracted to the lighted membrane and soon fed on the drop of solution.

LOCATION OF VIRUS IN BEET LEAFHOPPER

Midintestine, or Stomach. Experiments were performed to determine whether the curly-top virus was located in the midintestine, or stomach, of infective beet leafhoppers. The fore and hind intestine were torn from the alimentary canal in the dissections and the midintestine left intact. The number of stomachs per cubic centimeter of solution usually varied from 10 to 25, but sometimes as many as 60 were used. The crushed stomachs in the solution were either fed directly to previously noninfective nymphs or after filtering through a fine Berkefeld candle. The results of inoculating healthy sugar-beet seedlings with curly-top virus by previously noninfective nymphs fed on solution containing crushed midintestines from infective adults was as follows:

	Beets inoculated	Beets infected
Unfiltered solutions	204	8
Filtered solutions	240	9

Salivary Glands. A large number of experiments were conducted with different feeding solutions containing crushed salivary glands dissected from infective beet leafhoppers to determine whether the curly-top virus was located in these organs. In dissecting the adults, the head was severed from the thorax in sterile distilled water. If the heads were allowed to remain in the water for a few minutes the salivary glands protruded from the opening and could easily be dissected out with a small triangular scalpel. The number of salivary glands per cubic centimeter of solution varied from 15 to 25. Healthy sugar-beet seedlings were inoculated with curly-top virus by means of previously noninfective nymphs fed on unfiltered solutions containing these crushed salivary glands. Of the 352 beets thus inoculated, 39 developed curly-top.

TABLE 1

RESULTS OF INOCULATING HEALTHY SUGAR-BEET SEEDLINGS WITH PREVIOUSLY NONINFECTIVE BEET LEAFHOPPERS FED ON FEEDING SOLUTIONS INTO WHICH INFECTIVE LEAFHOPPERS HAD INJECTED SALIVA

Feeding solution	Infective nymphs fed feeding solution	Beets inoculated	Beets infected	Incubation period of disease in plant
	number	*number*	*number*	*days*
8 cc steam-extracted beet-root juice plus 8 cc 5 per cent beet-sugar solution	200-500	40	3	12, 13, 16
16 cc healthy beet-root juice	200-500	18	7	9, 12, 13, 14, 14, 14, 36
16 cc filtered healthy beet-root juice	200-500	18	0	......

Saliva. Experiments on the transmission of curly-top virus by previously noninfective beet leafhoppers which had fed on solutions in which saliva was injected by infective leafhoppers were made with three feeding solutions. The feeding equipment consisted of a small culture or "Esmarch" dish (50 x 10 mm) containing about 16 cc of solution (fig. 3) as described in a previous paper (Severin and Swezy, 1928). The results are indicated in table 1.

As in Carter's experiments, previously mentioned (p. 545), the incubation period of the disease in the beet was occasionally prolonged: in one beet it was 36 days, as compared with the usual period of 10 to 14 days under greenhouse conditions.

Blood. Attempts were made to determine whether the curly-top virus was located in the blood of infective beet leafhoppers. The aorta opens in the vicinity of the brain. Hence the leafhoppers were decapitated, and the blood was allowed to ooze into a drop of the culture medium. The solution consisted of sterile distilled water containing 5 per cent alcoholic pure sucrose. Blood from 25 infective beet leafhoppers was used for each cubic centimeter of solution. Previously noninfective nymphs were fed on the solution, both unfiltered and filtered, and then transferred to healthy beet seedlings. The results were as follows:

	Beets inoculated	Beets infected
Unfiltered solution	14	4
Filtered solution	50	1

Ovaries. Tests were made to determine whether the curly-top virus could be transmitted with the ovaries crushed in a solution. The blood, which contains the curly-top virus, bathes the ovaries; hence they were thoroughly washed in many changes of sterile, distilled water. The ovaries from 10 to 50 females were dissected and crushed in 1 cubic centimeter of solution. The microculture slide and drop methods of feeding were used. Previously noninfective nymphs after feeding on the culture solution containing the crushed ovaries failed to transmit curly-top virus to any of 112 beets in 56 feeding experiments.

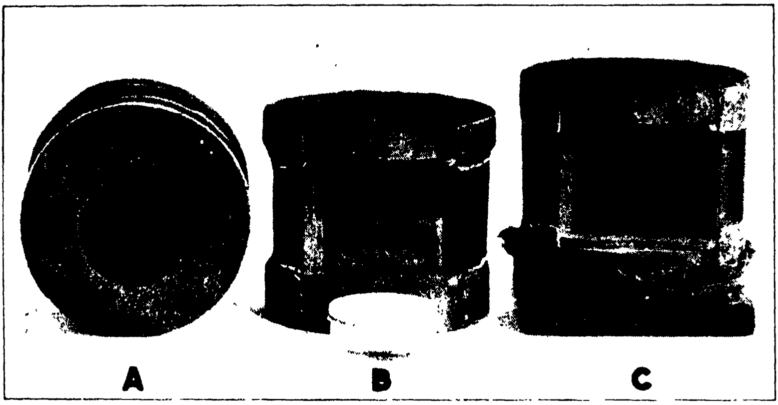

Fig. 3. Small culture or "Esmarch dish" containing feeding solution placed directly behind the glass in a cage lined with black sateen.

SUMMARY

Previously noninfective beet leafhoppers obtained the curly-top virus and transmitted the infective principle to healthy beet seedlings by feeding on solutions containing crushed stomach, salivary glands, saliva, and blood from infective leafhoppers and on the filtrates prepared with these organs. Negative results were obtained with the ovaries dissected from infective female leafhoppers.

The saliva containing the virus was injected into a feeding solution by infective beet leafhoppers and was recovered by previously noninfective insects and transferred to beet seedlings.

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SPINACH YELLOW DWARF

HENRY H. P. SEVERIN and DONALD H. LITTLE

SPINACH YELLOW DWARF[1]

HENRY H. P. SEVERIN[2] and DONALD H. LITTLE[3]

INTRODUCTION

FIVE VIRUS DISEASES of spinach (*Spinacia oleracea* L.) have thus far been reported to occur under natural conditions in the United States—namely, spinach blight (McClintock and Smith, 1918)[4], which is identical with common cucumber mosaic (Hoggan, 1933); aster yellows (Kunkel, 1926; Severin, 1934; Severin and Frazier, 1945); sugar-beet curly top (Severin and Henderson, 1928; Scott, 1935; Adams, 1936); beet mosaic (Jones, 1931; Hoggan, 1933; Smith, 1934); and spotted wilt (Gardner, Tompkins, and Thomas, 1937).

This paper deals with still another naturally occurring virus disease of spinach—spinach yellow dwarf. The aspects covered include symptoms and host range of the disease, the properties and aphid vector of the virus, transmission of the virus by single aphids, a comparison of the transmission of the virus by one species of aphid with mechanical inoculation, and retention of the virus by aphids.

MATERIALS AND METHODS

Source of Virus. The source of the spinach-yellow-dwarf virus was naturally infected spinach plants obtained from the truck-crop fields near San Pablo. The virus was retained through repeated mechanical inoculations and aphid transmission to spinach.

Spinach Extract. Juice from diseased spinach plants was obtained by grinding the plants to a pulp in a sterilized food chopper or mortar. The pulp was then placed between two layers of cheesecloth and the sap expressed by hand into a sterile glass dish.

Mechanical Inoculation. The method of mechanical inoculation used is that described by Rawlins and Tompkins (1936). After inoculation, the inoculum and the carborundum were washed from the leaves with water. The virus extract from each preparation was inoculated into 5 healthy spinach plants.

Variety of Spinach. Long Standing Bloomsdale spinach was used in studies of properties and aphid transmission of the virus and as a source of virus in host-range studies. This variety grows rapidly and remains in good condition a long time without bolting to seed.

Noninfective Aphids. Noninfective green peach aphids were obtained from populations reared on healthy sugar beets maintained in the greenhouse.

Methods of Transferring Aphids. High populations of aphids were transferred by cutting off leaves which bore large numbers of aphids, then placing the leaves on the inner or youngest leaves of another plant. Small

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[4] See "Literature Cited" for citations, referred to in the text by author and date.

numbers of aphids were transferred from plant to plant with a moistened camel's-hair brush.

SYMPTOMATOLOGY

The most conspicuous symptoms of the disease on naturally infected spinach are yellow blotches on the outer, or oldest, leaves, and dwarfed, puckered, curled, mottled inner, or youngest, leaves. Typical symptoms are illustrated in plate 1.

The first visible symptoms of the disease on spinach are a clearing of the veinlets (plate 2, *A*) and curvature of the midrib. These symptoms develop within from 12 to 14 days after inoculation in the greenhouse, and from 25 to 35 days out of doors during the winter.

The younger leaves next show yellow and green mottling, puckering, curling, and blisterlike elevations (plate 1). The older leaves show numerous small chlorotic areas (plate 2, *B*) which later coalesce and form conspicuous yellow blotches (plate 2, *C*). The yellow blotches become necrotic, the condition usually beginning at the basal margin of the leaf (plate 2, *D*) and progressing toward the tip. The heart of the infected plant is stunted, with shortened petioles on the younger leaves (plate 1).

As the disease progresses, the older leaves gradually become brown and dry. The young leaves usually remain mottled and distorted for a week or more after the older leaves are dead, but eventually they also become yellow and die.

HOST RANGE

Natural Infection. The natural host range of the yellow-dwarf virus so far known is limited to spinach. The virus was recovered from naturally infected spinach by inoculation of extracted sap into healthy spinach.

Experimental Infection. The following ten horticultural varieties of spinach have been experimentally infected with the virus by mechanical inoculation:

Chenopodiaceae:
  Spinacia oleracea, varieties Broad Flanders, Giant Thick Leaved, Juliana, King of Denmark, Long Standing Bloomsdale, Prickly Seeded Dark Green, Savoy Leaved or Bloomsdale, Thick Leaved Nobel, Virginia Savoy, and Virofloy.

The variety Virginia Savoy spinach is resistant to spinach blight (Smith, 1920; Hoggan, 1933), but is susceptible to the yellow-dwarf virus.

Recovery of Virus. The virus was recovered from all the experimentally infected varieties of spinach by inoculation of extracted sap in healthy spinach.

Nonsusceptible Plants. The following economic and ornamental flowering plants, tested by mechanical inoculation, are nonsusceptible to the disease:

Aizoaceae:
  Tetragonia expansa, New Zealand spinach
Campanulaceae:
  Campanula medium, canterbury bells
Chenopodiaceae:
  Beta vulgaris, varieties of garden beets: Crimson King, Crosby's Egyptian, Detroit Dark Red, Early Blood Turnip, Extra Early Flat Egyptian, Early Wonder, Ferry's Crosby, and Good for All

- *Beta vulgaris*, varieties of mangel-wurzel or stock beets: Danish Red Giant Eckendorf, Danish Sludstrup, Danish Yellow Giant Eckendorf, Giant Half Sugar Green Top, Giant Half Sugar Rose Top, Giant Yellow Intermediate, Mammoth Long Red, Yellow Leviathan
- *Beta vulgaris* var. *cicla*, varieties of Swiss chard: Large Ribbed Dark Green, Lucullus Dark Green

Compositae:
- *Callistephus chinensis*, aster
- *Lactuca sativa*, lettuce

Crucifereae:
- *Brassica oleracea* var. *capitata*, cabbage
- *Brassica campestris*, common yellow mustard
- *Mathiola incana* var. *annua*, stock or gilly flower

Cucurbitaceae:
- *Cucurbita pepo*, varieties of squash: Italian or Zucchini, White Bush Scallop
- *Citrullus vulgaris*, varieties of watermelons: Kleckey's Sweet, Klondike
- *Cucumus sativus*, White Spine cucumber

Geraniaceae:
- *Pelargonium zonale*, geranium

Leguminosae:
- *Lupinus hartwegii*

Malvaceae:
- *Gossypium hirsutum*, cotton, Acala variety

Nyctaginaceae:
- *Mirabilis jalapa*, four-o'clock

Primulaceae:
- *Primula veris*, cowslip

Solanaceae:
- *Capsicum frutescens*, California Wonder pepper
- *Lycopersicon esculentum*, Marglobe tomato
- *Nicotiana glutinosa*
- *Nicotiana alata* var. *grandiflora*, tuberous-flowered tobacco
- *Nicotiana tabacum*, Turkish tobacco
- *Petunia hybrida*, petunia, Rosy Morn variety
- *Datura stramonium*, stramonium
- *Solanum tuberosum*, potato

Umbelliferae:
- *Foeniculum vulgare* var. *dulce*, Florence fennel
- *Apium graveolens* var. *dulce*, celery, Golden Self-Blanching variety

The host range of the virus so far discovered is thus limited to varieties of spinach. Plants in only thirteen families were tested, however, and it may be possible that economic or ornamental plants or weeds in other families are susceptible. A limited host range for this virus would not be suprising, for those of other mosaic viruses are limited. For example, Tompkins and Middleton (1941) found that the host range of a mosaic disease of *Primula obconica* is limited to two additional species within the same genus, namely, *P. malacoides* and *P. sinensis.*

PROPERTIES OF THE VIRUS

Thermal Inactivation. The thermal inactivation of the spinach-yellow-dwarf virus was determined in the extracted sap from leaves of experimentally infected spinach. Ten cubic centimeters of diseased sap was poured into thin glass test tubes, which were plugged with cotton and submerged in a water bath maintained at the desired temperature by an electric thermostat. An agitator connected to an electric motor kept the water in circulation to maintain an evenly distributed temperature. The time of exposure in the water bath was 11 minutes, 1 minute being allowed for lag. After exposure at the desired temperature, the extract was cooled rapidly by partly submerging the test tubes in running tap water. Unheated controls were used in each test. Determinations were made at 5° C intervals. The results were as follows:

Temperature, ° C	Plants inoculated	Per cent infected
Unheated control	25	84
40	25	84
45	15	80
50	25	4
55	15	0
60	25	0
65	25	0
70	25	0

These data show that the virus remained active at 45° and that 1 infection was obtained from 25 inoculations at 50°; but no infections occurred at 55° to 70°.

Effect of Freezing Virus Extract. Two extractions of sap from leaves of experimentally infected spinach plants were placed in cold storage at –18° C immediately after extraction. Monthly inoculations of the virus extract in healthy spinach were made for a period of 6 months. The results were as follows:

Age of virus extract, months	Plants inoculated	Plants infected
Control	10	9
1	10	8
2	10	9
3	10	6
4	10	5
5	10	4
6	10	1

Thus freezing did not inactivate the virus at the end of 6 months, although only 1 infection was obtained with 10 plants inoculated at the end of this time.

Tolerance to Aging in Vitro. Tests were made to determine the inactivation of the virus in diseased spinach sap exposed to the air at room temperatures. Five cc of the expressed juice from experimentally infected spinach plants was poured into sterile test tubes which were plugged with cotton. Inoculations of the virus extract stored in test tubes were made at intervals of 24 hours for a period of 9 days. The following data were obtained, 25 plants being inoculated in each test:

Days exposed	Plants infected	Days exposed	Plants infected
0; Control	19	5	2
1	15	6	3
2	14	7	2
3	7	8	0
4	7	9	0

These data indicate that infectivity of the virus occurred after the extract was aged *in vitro* from 3 to 7 days and that no infections were obtained at the end of 8 and 9 days.

Tolerance to Dilution. In determining the tolerance of the virus to dilution, the juice expressed from experimentally infected spinach was diluted with sterile distilled water. The infections obtained were as follows:

Dilution	Plants inoculated	Per cent infected
Undiluted control	45	80
1:10	45	87
1:100	45	76
1:1,000	45	36
1:5,000	45	16
1:10,000	45	13
1:15,000	35	3
1:20,000	35	6
1:25,000	35	0
1:50,000	45	0

The tolerance to dilution was 1:20,000. There is considerable variation in the tolerance to dilution of the virus with different extractions; with three virus extracts, infections were obtained at dilutions of only 1:100.

APHID TRANSMISSION OF VIRUS

By Green Peach Aphid. Large populations of the green peach aphid, *Myzus persicae* (Sulzer) have been observed in the fields near San Pablo. This aphid transmitted the spinach-yellow-dwarf virus from experimentally infected to healthy spinach plants in the greenhouse.

By Single Aphids during Short Feeding Time. An experiment was conducted to determine whether single green peach aphids were able to transmit the virus during short feeding periods. Single previously noninfective wingless aphids were fed 5 minutes on a diseased plant and then 5 minutes on a healthy spinach plant. Two infections were obtained with 25 aphids tested.

Comparison of Transmission of Virus by Aphids with Mechanical Inoculation. Transmission of the virus by 20 green peach aphids was compared with that by mechanical inoculation. After a large population of previously noninfective green peach aphids was reared on experimentally infected spinach plants, lots of 20 aphids were transferred from diseased plants to each of 5 healthy spinach plants. The extract from each diseased plant on which the aphids fed was also inoculated into 5 healthy spinach plants. The results were as follows:

Test no.	Plants infected by aphid transmission	Plants infected by mechanical inoculation
1	4	5
2	4	3
3	2	4
4	0	4
5	0	3
Total	10	19

Infections obtained by aphids thus averaged 40 per cent and by mechanical inoculation 76 per cent.

Retention of the Virus. Two experiments were conducted to determine the length of time the green peach aphids would retain the virus. In the first experiment, 5 lots of 20 wingless green peach aphids were fed upon diseased spinach plants for a period of 3 days, and then each lot was transferred daily to 3 successive healthy spinach plants. The aphids remained on the third plant for a period of 1 week. The results were as follows, the plus sign indicating production of the disease and the minus sign indicating that no disease resulted:

Test no.	1st day	2d day	3d day
1	+	–	–
2	+	–	–
3	+	–	–
4	+	–	–
5	+	–	–
Total +	5	0	0
Total –	0	5	5

Thus aphids infected only those plants fed upon the first day.

In the second experiment, 5 lots of 20 wingless aphids were fed upon diseased spinach plants for a period of 3 days, and then each lot was transferred hourly to 10 successive healthy spinach plants. As appears in table 1, 5 lots of aphids infected a spinach plant during the first hour, and 2 lots during the second hour also, and none thereafter.

TABLE 1

RETENTION OF SPINACH-YELLOW-DWARF VIRUS BY LOTS OF 20 GREEN PEACH APHIDS, *Myzus persicae*, TRANSFERRED HOURLY TO 10 SUCCESSIVE HEALTHY SPINACH PLANTS

Test no.	Results* on successive plants, with hourly transfers									
	1st	2d	3d	4th	5th	6th	7th	8th	9th	10th
1	+	+	–	–	–	–	–	–	–	–
2	+	+	–	–	–	–	–	–	–	–
3	+	–	–	–	–	–	–	–	–	–
4	+	–	–	–	–	–	–	–	–	–
5	+	–	–	–	–	–	–	–	–	–
Total +	5	2	0	0	0	0	0	0	0	0
Total –	0	3	5	5	5	5	5	5	5	5

* The plus sign (+) indicates the production of the disease, and the minus sign (–) shows that no disease resulted.

DESCRIPTION OF SPINACH-YELLOW-DWARF VIRUS

Name: Spinach yellow dwarf.

Host plant: Spinach (*Spinacia oleracea*).

Symptoms of disease: Vein clearing, yellow and green mottling, puckering, curling, blisterlike elevation, curvature of midribs and petioles, and stunting of young leaves. Small chlorotic areas, which fuse, forming yellow blotches, followed by necrosis and drying of outer leaves. Yellowing and death of younger leaves.

Incubation period of disease: **25 to 30 days out of doors during winter.**

Properties of virus: **Thermal inactivation 55° C in 10-minute exposures, resistance to aging *in vitro* 8 days, tolerance to dilution 1:20,000.**

Modes of transmission: **Natural inoculation by green peach aphid, *Myzus persicae;* mechanical inoculation with expressed juice.**

SUMMARY

The symptoms of yellow dwarf on naturally and experimentally infected spinach are described.

The host range of this virus is limited to spinach. Twenty-six species of plants in 23 genera belonging to 13 genera, were nonsusceptible to the virus. Some of the properties of the spinach-yellow-dwarf virus are summarized as follows: thermal inactivation of the virus was 55° C in 10-minute exposures. Freezing sap extracted from diseased spinach kept in cold storage at –18° C did not inactivate the virus at the end of 6 months. An inactivation of the virus occurred after the virus extract was exposed to the air at room temperature for a period of 8 days. The tolerance to dilution of extracted diseased spinach juice was 1 :20,000.

The green peach aphid, *Myzus persicae* (Sulzer), was demonstrated to be a vector of the virus. Two of 25 previously noninfective wingless aphids transmitted the virus after feeding 5 minutes on a diseased plant and 5 minutes on each healthy plant. Infections obtained by lots of 20 aphids averaged 40 per cent, and by mechanical inoculation of the virus extract from each plant on which the aphids had fed, 76 per cent. Lots of 20 wingless aphids transmitted the virus from diseased to healthy plants during the first day, but failed to infect plants during the second day and during the following week. Five lots of 20 infective aphids, when transferred hourly to successive sets of healthy spinach for a period of 10 hours, infected 5 plants during the first hour, 2 plants during the second hour, and none thereafter.

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PLATES

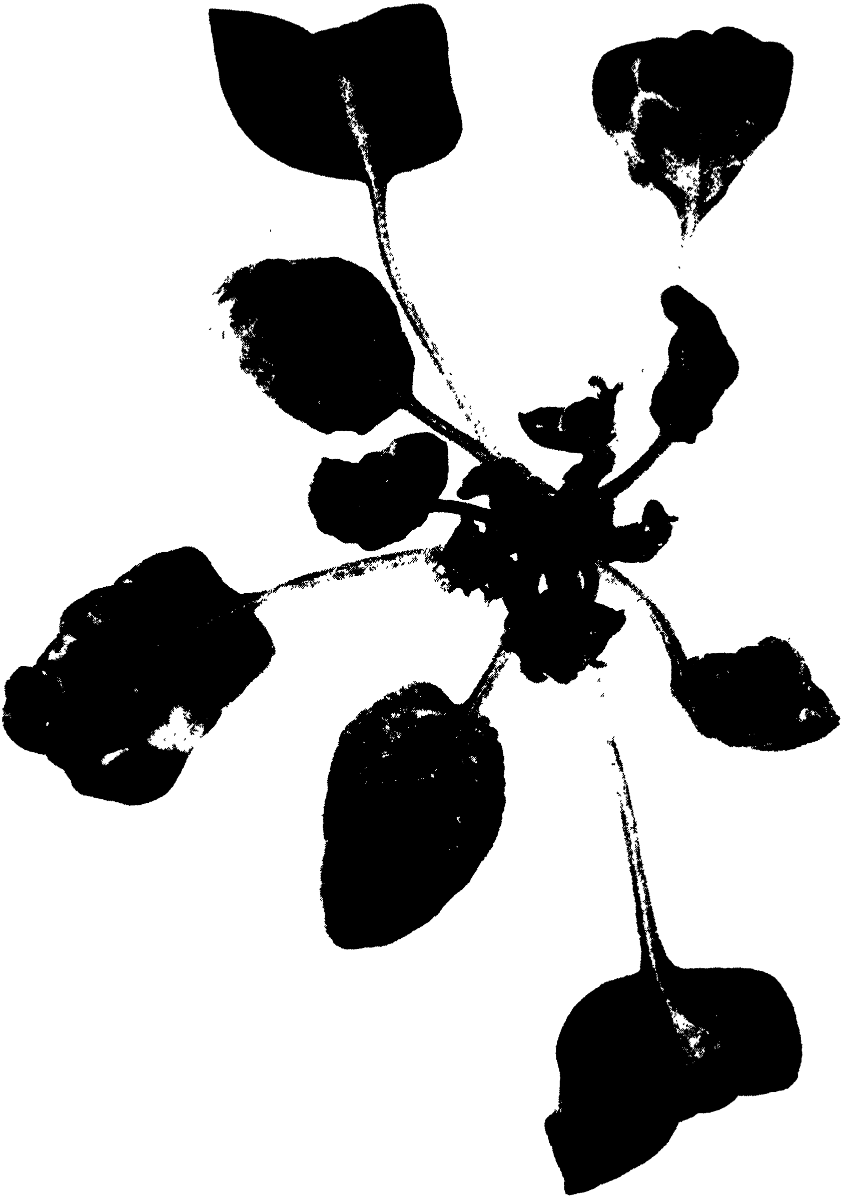

Plate 1.—King of Denmark spinach (*Spinacia oleracea*) experimentally infected with yellow-dwarf virus showing yellow blotches on the older, puckered, and curled leaves; and showing dwarfed, puckered, mottled, younger leaves, some displaying curved petioles.

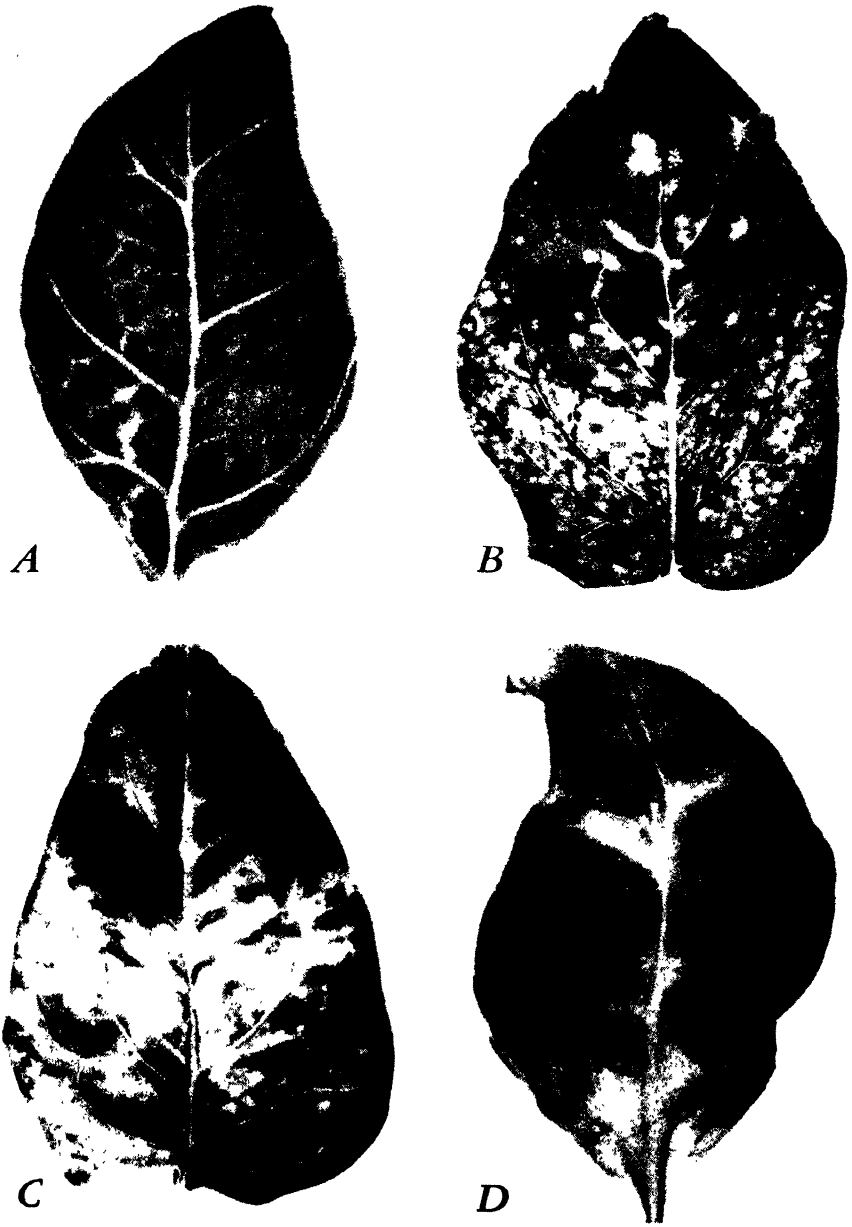

Plate 2.—King of Denmark spinach (*Spinacia oleracea*) infected with yellow-dwarf virus: *A*, cleared veinlets on youngest leaf; *B*, leaf showing numerous, small, circular, chlorotic areas, *C*, yellow blotches formed by fusion of the chlorotic areas; *D*, chlorosis and necrosis of leaf.

WEEDS EXPERIMENTALLY INFECTED WITH BEET-MOSAIC VIRUS

HENRY H. P. SEVERIN and ROGER M. DRAKE

WEEDS EXPERIMENTALLY INFECTED WITH BEET-MOSAIC VIRUS[1]

HENRY H. P. SEVERIN[2] and ROGER M. DRAKE[3]

INTRODUCTION

IT IS IMPORTANT to know what plants growing in the cultivated areas and on the uncultivated plains and foothills are reservoirs of the beet-mosaic virus. After the pasture vegetation becomes dry on the plains and foothills, enormous flights of aphid vectors fly into the cultivated areas. and are often abundant on favorable weeds, varieties of beets, and other economic plants. Unpublished data indicate that the host range of the virus among economic plants is limited to plants belonging to the families of Azioaceae, Chenopodiaceae, and Papaveraceae.

This paper deals with the weed host range of the beet-mosaic virus. A study was made of the sequence of symptoms of experimentally infected weeds so that naturally infected plants could be recognized in the field. Some reports on weeds susceptible to the virus have been published; these are discussed in connection with our results (pp. 570–571.)

METHODS

Weeds grown from seeds were experimentally infected with the virus by mechanical inoculation using the carborundum method described by Rawlins and Tompkins (1936).[4] The virus was recovered from each species of weed and transferred to sugar beets by the same method.

WEED HOST RANGE AND SYMPTOMATOLOGY

Six species of weeds in three genera of the family Chenopodiaceae were experimentally infected by mechanical inoculation with the virus extract from mosaic-infected beets. Systemic infection occurred in all of the weeds from which the virus was recovered. The symptoms on the weeds experimentally infected with the virus are as follows.

Bractscale. The symptoms on bractscale, *Atriplex bracteosa,* are cessation of growth and a bending and curling of the apical shoot (plate 1, *B*) on infected plants. The young leaves are dwarfed, cupped outward, occasionally twisted along the midrib (plate 1, *C*) sometimes asymmetrical, mottled with small chlorotic spots; later the leaves become necrotic. Necrosis of the young leaves and death of the apical shoot occur within 3 weeks after inoculation.

Red Orache, or Redscale. The symptoms on red orache, *Atriplex rosea,* develop in essentially the same manner as those described on bractscale. All of the infected plants died within 3 weeks after inoculation.

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[1] Received for publication December 21, 1946.

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[4] See "Literature Cited" for citations, referred to in the text by author and date.

Spear Orache, or Spearscale. On spear orache, *Atriplex patula* var. *hastata,* small, chlorotic spots appear on the youngest leaves and these gradually enlarge on the somewhat older leaves (plate 2, *D*).

Lamb's-Quarters, or White Pigweed. The onset of mosaic symptoms on lamb's-quarters, *Chenopodium album,* is marked by dwarfed leaves with chlorotic spots and retarded growth of the apical shoots. The apical shoots are bent (plate 1, *F*, *G*), the youngest leaves curled, and cupped outward. Mottling develops on the youngest and older leaves (plate 1, *G*). Some leaves are not mottled but show numerous chlorotic rings surrounding green tissue (plate 1, *E*); later the rings become necrotic. Young leaves on the apical shoots become necrotic and dry (plate 1, *F*, *G*), and eventually the entire plants are killed.

This confirms the findings of Smith (1937), who recorded lamb's-quarters as a host plant of beet mosaic in England and described the symptoms on this weed.

Sowbane, or Nettle-Leaf Goosefoot. The first visible symptom of the disease on sowbane, *Chenopodium murale,* are cleared veins and veinlets (plate 2, *C*, lower row) followed by small, chlorotic areas on young leaves of the apical and axillary shoots (plate 2, *A*, *B*). Growth is retarded and the leaves become twisted, curled outward, and sometimes asymmetrical (plate 2, *A*, *B*). Necrosis of isolated spots or large chlorotic areas results in the death of the young leaves on both apical and axillary shoots, and necrotic streaks extend down the stem in the later stage of the disease (plate 2, *B*). Death of infected plants usually occurs within 4 weeks or less after inoculation.

Russian Thistle. The first reliable symptom on seedlings of Russian thistle, *Salsola kali* var. *tenuifolia,* was stunting of the apical and axillary shoots. Small, necrotic, sunken areas on the needlelike leaves, giving a beaded appearance, soon cause death and blackening of the shoots (plate 2, *E*). Necrotic streaks develop on the stem, and the infected seedlings die within 2 weeks after inoculation.

NONSUSCEPTIBLE WEEDS

The following weeds are nonsusceptible to beet mosaic. All inoculated weeds which failed to show symptoms of the disease were tested for the recovery of the virus.

Compositae, sunflower family: common sow thistle, *Sonchus oleraceus;* prickly sow thistle, *S. asper*

Cruciferae, mustard family: common yellow mustard, *Brassica campestris*

Malvaceae, mallow family: dwarf mallow, *Malva rotundifolia*

Plantaginaceae, plantago family: common plantain, *Plantago major*

Polygonaceae, buckwheat family: curly dock, *Rumex crispus;* fiddle dock, *R. pulcher;* sheep sorrel, *R. acetosella*

Solanaceae, nightshade family: black nightshade, *Solanum nigrum;* stramonium, *Datura stramonium.*

A number of investigators have reported the transmission of the beet-mosaic virus by means of aphids or mechanical inoculation to a number of weeds in many families contrary to the results of the present investigations.

Novinenko (1930) states that *Aphis fabae* Scopoli was able to transmit the beet-mosaic virus to *Amaranthanus retroflexus* (Amaranthaceae), *Chenopodium album* (Chenopodiaceae), and *Sonchus arvensis* (Solanaceae).

Our investigations failed to show that common sow thistle, *Sonchus oleraceus,* and prickly sow thistle, *S. asper* (Solanaceae), were susceptible to beet mosaic, nor could the virus be recovered from these weeds.

Verplancke (1933) reports that forty-five species of weeds in twenty-two families harbored the virus, but his investigations on the weed-host range were discredited by Quanjer and Roland (1936) and Quanjer (1936).

A number of species of aphids have been found in nature in the United States, on weeds susceptible to the virus. Essig (1926) reports that the bur clover or cowpea aphid, *Aphis medicaginus* Koch, was taken on lamb's-quarters, *Chenopodium album.* Gillette and Palmer (1931-1934) collected *A. medicaginus* and the green peach aphid, *Myzus persicae* (Sulzer) on lamb's-quarters and on Russian thistle, *Salsola kali* var. *tenuifolia. Aphis medicaginus* and *Myzus persicae* have been demonstrated to be vectors of the beet-mosaic virus to economic plants (unpublished data) and in all probability are vectors of the virus to susceptible weeds under natural conditions.

SUMMARY

Six species of weeds in three genera in the family Chenopodiaceae were experimentally infected by mechanical inoculation with the virus extract from sugar-beet mosaic. Systemic infection resulted in all of the weeds from which the virus was recovered. The species infected were as follows:

Bractscale, *Atriplex bracteosa*
Red orache, or redscale, *Atriplex rosea*
Spear orache, or spearscale, *Atriplex patula* var. *hastata*
Lamb's-quarters, or white pigweed, *Chenopodium album*
Sowbane, or nettle-leaf goosefoot, *Chenopodium murale*
Russian thistle, *Salsola kali* var. *tenuifolia*

Nine species of weeds in the families Compositae, Cruciferae, Malvaceae, Plantaginaceae, Polygonaceae, and Solanaceae were nonsusceptible to the virus.

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PLATES

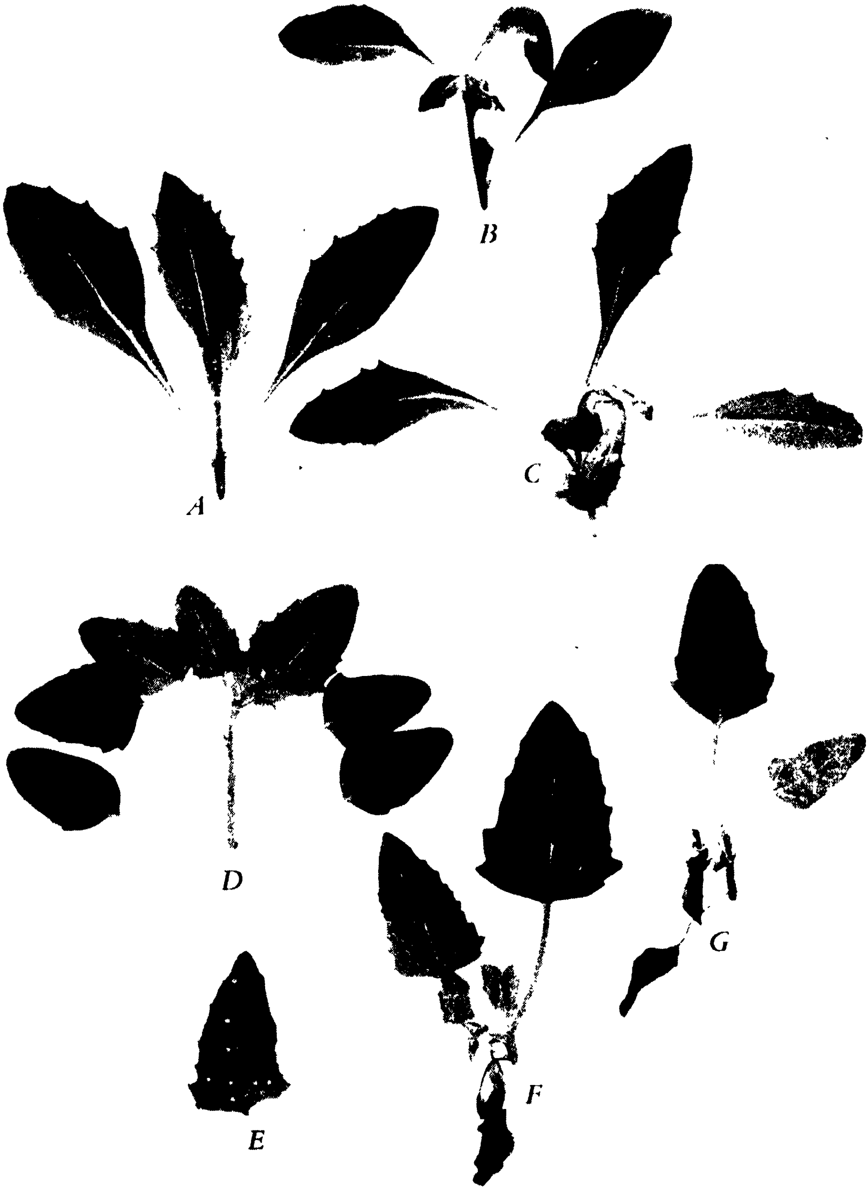

Plate 1.—*A–C*, Bractscale, ***Atriplex bracteosa:*** *A*, apical shoot from healthy check or control plant; *B*, *C*, apical shoots from plants experimentally infected with beet-mosaic virus, *B* showing bending and curling of dwarfed, youngest leaves, and *C* showing outward-cupped younger leaves and leaf twisted along the midrib. *D–G*, Lamb's-quarters, or white pigweed, ***Chenopodium album*** : *D*, apical shoot from check or control plant; *E–G*, leaves and shoots from plants experimentally infected with beet-mosaic virus, *E* showing chlorotic rings surrounding green tissue on young leaf, *F* showing bending of apical shoot, dwarfed youngest leaves, and dead leaves; and *G* showing necrotic and dead youngest leaf on apical shoot and mottling of older leaf.

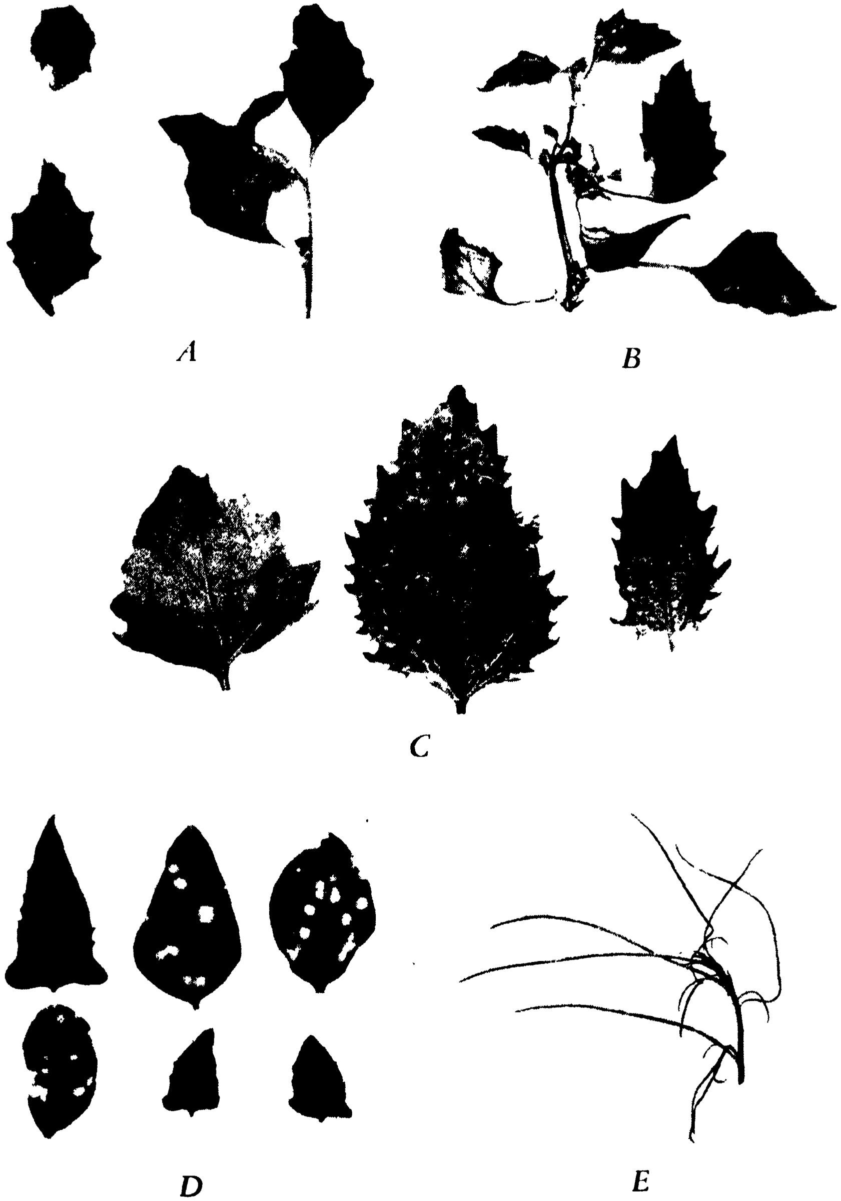

Plate 2.—Weeds experimentally infected with beet-mosaic virus: *A–C*, Sowbane, or nettle-leaf goosefoot, *Chenopodium murale*: *A*, left, two leaves showing chlorotic spots; right, bending of apical shoot with outward-cupped and twisted, yellow-spotted leaf; *B*, bending of apical end of branch and axillary shoots with dwarfed, curled, or twisted leaves, chlorotic areas on older leaves, and other leaves necrotic and dead, and with black, necrotic streaks extending along the stems and axillary shoots; *C*, left, leaf from check or control plant, two others showing mottling and chlorotic spots. *D*, Spear orache or spear scale, ***Atriplex patula*** var. ***hasta***: upper row, left, leaf from check or control plant, all others showing chlorotic spots. *E*, Russian thistle, ***Salsola kali*** var. ***tenuifolia***, shoot showing necrotic, black, axillary leaves and necrotic streaks on the stem.

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VIRUSES THAT INDUCE BREAKING IN COLOR OF FLOWER PETALS IN PANSIES AND VIOLAS[1]

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INTRODUCTION

PANSIES (*Viola tricolor* var. *hortensis*) and violas, or tufted pansies (*Viola cornutus*) in California home gardens, nurseries, and seed farms frequently show a conspicuous breaking in the color of flower petals. The flowers, especially violas, are often dwarfed and malformed. Such plants are frequently stunted. An investigation was undertaken to determine the cause.

When the trouble was found to be due to two viruses—celery-calico and western-cucumber-mosaic—the method of transmission and control of the diseases were investigated. A study was made of the symptomatology on pansies and violas infected with these viruses and with common-cucumber-mosaic virus (which has not been reported in California). Experiments were also conducted with viruses known to cause breaking in flower petals of other ornamentals, to determine whether any of these would induce the disease in pansies and violas. This paper reports the results of these investigations.

A review of the literature indicates that three references to a mosaic disease in species of *Viola* in the United States have appeared. Martin[3] (1926) mentions a "yellows—undetermined mosaic type" on pansy (*Viola tricolor*) in Washington, D.C. Edson and Woods (1936) report a mosaic on *Viola* sp. in the state of Washington. Perone (1939) lists pansy among twenty-nine ornamental flowering plants affected with mosaic in New Jersey, and in a general description of symptoms reports "breaking of blossoms on these host plants."

In England, Smith (1935, 1936*a*, 1936*b*, 1937) reported breaking in violas and stated that inoculation experiments "seem to show that the virus causing this variegation is a strain of cucumber-mosaic virus (cucumber virus I)." Moore (1933–1942) reported a mosaic on several pansies and violas in Somerset in 1936.

In New Zealand, Chamberlain (1936) described foliage symptoms and breaking in pansy, viola, and violet.

In New South Wales, Dunn (1941), in notes contributed by the biological branch, recorded for the first time a "mosaic (virus) of pansy (*Viola tricolor*) in Sydney, Metropolitan area."

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[1] Received for publication December 30, 1946.

[2] Entomologist in the Experiment Station.

[3] See "Literature Cited" for data on citations, referred to in the text by author and date.

MATERIALS AND METHODS

Sources of Viruses. The principal sources of the celery-calico virus were naturally infected pansies and violas. Infected pansies were collected in nurseries and gardens in Berkeley, Albany, El Cerrito, San Pablo, and Salinas. Infected Papilio, Radio, Blue Perfection, and unknown varieties of violas were obtained in Berkeley, San Pablo, and Salinas. The virus was identified by inoculating celery with the virus extract from naturally infected pansies and violas; the celery developed typical symptoms. Later, celery and other host plants were used as virus sources. The virus was maintained by repeated mechanical inoculation in and aphid transmission to pansies and violas.

The original source of most of the western-cucumber-mosaic virus used in experimental work was an infected Honey Dew melon plant (*Cucumis melo* var. *inodorus*) collected on November 7, 1932, at Keyes, in the San Joaquin Valley. The virus was maintained by repeated mechanical inoculation of various host plants, and also the virus extract was kept in cold storage at −18° C. After the experimental work was started, natural infection of pansies and violas growing in the San Joaquin Valley was demonstrated.

Common-cucumber-mosaic virus was kindly sent to me by James Johnson, University of Wisconsin.

Virus Extract. In preparation of juice from the leaves of pansies, violas, and other host plants, the leaves were washed in distilled water and ground to a pulp in a mortar. The pulp was placed in two layers of cheesecloth, a thick jellylike juice was pressed out by hand. This juice was diluted with sterile, distilled water.

Centrifugation. The diluted, extracted juice was centrifuged for 1 hour at a speed of 3,500 revolutions per minute.

Mechanical Inoculation. The carborundum method of mechanical inoculation described by Rawlins and Tompkins (1936) was used. Shortly after inoculation, the carborundum and inoculum were washed from the leaves with water.

Aphids. The methods of rearing and transferring aphids and the production of noninfective aphids have been described in a previous paper (Severin and Freitag, 1938).

CELERY-CALICO VIRUS

The celery-calico virus is a cucumber-mosaic virus which is common in the coastal fog belt and also occurs in the hot interior regions of California. Celery calico has been found in all of the large celery districts of this state (Severin and Freitag, 1938). The geographical distribution of the celery-calico virus includes California (Severin and Freitag, 1938), Washington, and Idaho (Severin, 1942*a*).

Transmission from Naturally Infected Host Plants. The celery-calico virus was transmitted by mechanical inoculation to pansies and violas from the following naturally infected host plants:

Chenopodiaceae:
  Spinach (*Spinacia oleracea*)
Cucurbitaceae:
  Cucumber (*Cucumis sativus*)
  Squash (*Curcurbita* sp.)

Ranunculaceae:
  Perennial delphinium (hybrids and horticultural varieties)
  Larkspur (*Delphinium ajacis*)
Solanaceae:
  Tomato (*Lycopersicon esculentum*)
Umbelliferae:
  Celery (*Apium graveolens* var. *dulce*)
Violaceae:
  Pansy (*Viola tricolor* var. *hortensis*) and viola (*V. cornuta*)

The virus was recovered from a large number of naturally infected varieties of pansies and violas collected in the East Bay districts and transmitted to healthy pansies and violas grown from seeds.

Symptoms on Pansies. The foliage symptoms of celery calico on naturally infected pansies are not conspicuous in the early stages of the disease. The first symptom on the youngest leaves is a clearing of the veins and veinlets. The youngest leaves are dwarfed and malformed. When a large number of infected pansies are examined, an occasional plant shows typical symptoms resembling celery calico (Severin and Freitag, 1938). Some of the older leaves of such a pansy are amber yellow except for small green areas enclosed in yellow veins and veinlets. In the later stages of the disease, the plant turns yellow. Young infected plants are stunted with shortened internodes and plants finally die.

The color changes involved in breaking of flower petals vary according to the normal color of the pansies. Red varieties develop red streaks, which later become yellow and then white. Purple varieties show purple streaks and later white streaks. Dark-blue and pale-blue varieties develop dark-blue and pale-blue streaks followed by white streaks. So-called black varieties show black streaks, which turn purple, and then white. All of the petals may show breaking, or all but the lowest, or only the two upper petals.

Pansies infected in the flats, when transplanted in the home gardens, develop malformed and dwarfed flowers. Pansies infected when older may continue to blossom and develop ruffled flowers or flowers with the margins rolled inward. Frequently breaking occurred on one or more branches while on other stems on the same plant the flowers were normal.

A brief description of the early and late symptoms on flowers of varieties of French bedding pansies follows:

Adonis: Purple streaks appear on the upper two petals and on the margins of the remaining petals; later, the purple streaks become white on the pale-blue flower.

Victoria: The petals show pale-red streaks; later, turn yellow, and then white.

Emperor William: The petals develop dark-blue streaks, which later become white and are more pronounced on the lower surface of the dark navy-blue flower.

King of the Blacks: The petals first show black streaks, which become purple. Blotches of purple appear on the petals; later, the streaks become white.

Light Blue: Pale-blue streaks alternating with white streaks occur on all petals; later, the two upper petals become blanched.

Lord Beaconsfield: The two upper petals are white with purple streaks on the lavender, heliotrope, and purple flowers.

Prince Henry: Purple and white streaks occur on the two upper petals, which later become blanched on the dark-blue flower.

Breaking in color of the flower petals in varieties of Giant Mastodon pansies are briefly described as follows:

Geneva Giant mixed: Short yellow or white streaks occur in the margin of the petal of the many-colored flowers.

Madame Steele: White streaks or bands appear on the two lateral and the lowest petals. The flowers are malformed.

Sea Blue: Numerous short white streaks develop on all petals.

Vulcano: Yellow alternating with red streaks occur on the petals.

Swiss Giant pansies inoculated with the virus showed the following symptoms on the flowers:

Dark Blue: Short white streaks occur on all petals.

Dark Red: The early flower symptoms consist of a few yellow streaks alternating with dark red; later white streaks alternate with pale-red streaks.

Lake of Thun, or Ullswater: White streaks appear on all petals, and later, blanching occurs on the blue flower with a blue-black center.

Infected Mastodon Jumbo pansies show a wide range of colors, shades, and markings (plate 1). The golden-yellow strains develop yellow streaks which later become white. Infected blue and yellow Jumbo pansies have blue, purple, and yellow bands. The brown pansy shows yellow and brown bands. The purple strain shows pale- and dark-purple streaks. White streaks appear on the petals of the blue strain.

Symptoms on Violas. When Papilio viola was inoculated with the sap expressed from perennial delphinium naturally infected with the celery-calico virus, the earliest foliage symptom was a clearing of the veinlets usually appearing at the base of the youngest leaves (plate 2, *B*) and sometimes spreading to the remaining portion of the leaves (plate 2, *C*). Later small chlorotic circular areas (plate 2, *D*) sometimes appear on the younger leaves. The oldest leaves develop lemon-yellow blotches (plate 2, *E*) which coalesce and spread over the entire leaves (plate 2, *F*). In the advanced stage of the disease, the plants are stunted with short upright stems bearing thick, linear leaves, frequently mottled with dark-green areas.

Of all varieties of violas examined in nurseries, Papilio violas show the most severe breaking in the color of the petals (plate 3, *B*). The flowers are dwarfed and malformed, with numerous white streaks (plate 3, *C*).

An intensive study was made of breaking in the petals of Radio viola. Conspicuous white streaks appear on some flowers (plate 4, *B*), followed by blanching with a few streaks of the normal color of the flower (plate 4, *C, D, E*) and finally the flower becomes pale blue (plate 4, *F*) and then white without streaks. Blotching may appear on some flowers (plate 4, *G, H*), followed by streaking (plate 4, *I, J*), and blanching (plate 4, *K*). The flowers may be dwarfed, malformed, and have white streaks (plate 4, *L*).

In some varieties of violas, the first symptom to appear is white streaks. In other varieties, the streaks are paler than the normal color of the variety. Blotches of white often appear in the normal color. Blanching of the flowers frequently occurs, with pale streaks or bands of the normal color.

A brief description of breaking in varieties of violas inoculated with celery calico follows:

Blue Perfection: White streaks occur on all petals.

Chantreyland: The apricot petals show pale-yellow streaks on the upper and lateral petals.

Heavenly Blue: White streaks appear on the petals; later, the petals become pale blue and then white.

Jersey Gem: A slight breaking in color of the violet-purple petals occurs

Lilac: White streaks appear on all petals; later, the petals become blanched with purple streaks.

Tufted "Pansy Violet": The flowers are dwarfed.

Papilio: Numerous white streaks develop on the blue petals (plate 3, *B*). The flowers are frequently dwarfed and malformed (plate 3, *C*).

Papilio Violet Lilac: White streaks occur on all blue petals.

Purple King: Pale-purple streaks develop in the pale-purple color of the flower.

Radio: Blotches of white with pale-blue streaks appear on the blue petals (plate 4, *G, H, I, J, K*); later, white streaks occur between the veins (plate 4, *B, L*) and finally the pale-blue streaks along the veins become broken (plate 4, *C, D, E*), and then disappear, and the petals become pale blue (plate 4, *F*) or white.

Rose Queen: White streaks and bands occur on all petals.

Royal Blue: Pale-blue streaks develop on the petals.

Recovery of Virus. The celery-calico virus was recovered from experimentally infected varieties of pansies and violas (with one exception) by mechanical inoculation of the extracted, centrifuged juice into celery and cucumbers. (The virus was transferred more readily to cucumbers than to celery.) Jersey Gem violas infected with celery-calico virus developed breaking in color of the flower petals, but numerous attempts made to recover the virus by mechanical inoculation yielded negative results.

WESTERN-CUCUMBER-MOSAIC VIRUS

Western cucumber mosaic occurs only in the interior regions of California and not in the coastal fog belt.

Transmission from Naturally Infected Host Plants. The host plants naturally infected with western-cucumber-mosaic virus include the following plants in four families:

Chenopodiaceae:
  Sugar beet (*Beta vulgaris*)
  Swiss chard (*Beta vulgaris* var. *cicla*)
  Spinach (*Spinacia oleracea*)
Cucurbitaceae:
  Cucumber (*Cucumis sativus*)
  Honey Dew melon (*Cucumis melo* var. *inodorus*)
Solanaceae:
  Tomato (*Lycopersicon esculentum*)
Umbelliferae:
  Celery (*Apium graveolens* var. *dulce*)

Symptoms on Pansies. The symptoms of western cucumber mosaic on pansies and violas cannot be distinguished from those induced by the celery-calico virus. The identity of each virus can only be determined by symptoms produced on other host plants.

A brief description of breaking in color of flower petals in varieties of French bedding pansies follows:

Adonis: The veins of the two upper and two lateral petals are purple with ashy-gray interveinal areas on the pale-blue flowers.

Emperor William: Numerous white streaks develop in the dark navy-blue petals.

King of the Blacks: Usually purple bands alternating with black streaks appear on the petals.

Light Blue: The veins are pale blue, with interveinal white areas; later the upper petals become blanched and all petals are ruffled.

Lord Beaconsfield: The two upper petals become blanched with purple veins, white blotches appear on the two lateral petals with purple veins, and a white blotch appears at the basal central margin, with purple veins near the margin of the basal petal of the lavender, heliotrope, and purple flower.

Prince Henry: The dark-blue flowers usually show diffuse blue streaking or blotches on all petals when severely affected by the disease. Such flowers are dwarfed, and their petals are curled, with the margins rolled inward.

The symptoms on varieties of Giant Mastodon pansies inoculated with the western-cucumber-mosaic virus follow:

Giant Geneva mixed: White streaks appear on the petals of the many-colored flowers.

Light Blue: The upper petals become blanched; on all other petals the veins are pale blue with interveinal white areas. The blossoms are dwarfed with ruffled petals.

Madame Steele: White streaks develop on the petals.

Sea Blue: White streaks are occasionally conspicuous on the lower surface of the flower; other flowers on the same plant may be normal.

Vulcano: Yellow streaks alternating with red streaks occur on the upper two petals.

The symptoms on inoculated Swiss Giant pansies are as follows:

Dark Blue: The petals develop white streaks, and the margins are rolled inward.

Dark Red: Yellow or white streaks appear on the upper and lateral petals.

Lake of Thun: The petals show white streaks and later blanching (plate 5, *H*) on the blue flower with a black center.

Symptoms on Violas. The symptoms on inoculated varieties of violas, briefly described, are as follows:

Blue Perfection: The flowers are dwarfed with white streaks on all petals.

Chantreyland: The upper and lateral petals of this apricot variety fade to a yellow color with a few white streaks on the upper petals.

Heavenly Blue: White blotches followed by white streaks appear on the upper petals and white bands on the lateral petals (plate 5, *B*).

Jersey Gem: The margins of the petals are curled inward, and a few white streaks may appear on the violet-blue petals.

Lilac: White streaks develop on the petals, and later blanching (plate 5, *E*, *J*).

Papilio: White blotches, streaks, and bands occur on the blue petals. The flowers are dwarfed, with the margins of the petals curled inward. The symptoms induced by the western-celery-mosaic and celery-calico viruses are identical on this variety of viola since all gradations of blotches, streaks, and bands occur on plants infected with these viruses.

Papilio Violet Lilac: White streaks occur on all petals.

Purple King: Dark-purple streaks appear with interspaces or blotches of pale purple. The deep-blue flowers are dwarfed with the two upper and lower petals cupped inward.

Rose Queen: The flowers are dwarfed with short white streaks on the petals.

Tufted "Pansy Violets": The plants are difficult to infect by mechanical inoculation and rarely does a flower show breaking (plate 5, *D*, *G*). The virus was rarely recovered; only 1 of 30 Zucchini squash plants developed symptoms of western cucumber mosaic with six virus extractions.

Recovery of Virus. The virus of western cucumber mosaic was readily recovered from all varieties of pansies, with the exception of Adonis, by inoculating cucurbits, but difficulty was experienced in recovering the virus from violas. Although no symptoms were observed on Victoria Blood Red pansy, the virus was recovered. The virus was recovered from all varieties of violas, after many trials with some varieties, and transferred to cucumbers and Zucchini squash.

COMMON CUCUMBER MOSAIC

Common cucumber mosaic is a destructive disease of cucumbers and other host plants in middle western and eastern United States, but is not known to occur in California.

In many of the varieties of pansies and violas tested, no flower symptoms appeared even though repeated inoculations were made with both infected cucumbers and *Nicotiana glutinosa* as sources of virus. The virus was not recovered from such plants.

Symptoms on Pansies. The first symptom to appear on some varieties of pansies and violas is a clearing of the veins and veinlets on the youngest leaves. Later, infected plants are stunted with shortened internodes. The older leaves are mottled and the younger leaves are small and narrow and turn yellow in the later stage of the disease. A brief description of flower breaking in varieties of inoculated pansies follows:

Lord Beaconsfield: The petals show dark-purple streaks with pale-purple bands between them on the lavender, heliotrope, and purple flower.

Madame Steele: Pale-purple streaks develop which appear as white streaks on the lower surface of the petals.

Lake of Thun, or Ullswater: Short white streaks appear on the buds and on all petals after the bud expanded (plate 6, *D*) of the blue flower with a blue-black center.

Symptoms on Violas. A brief description of breaking in inoculated varieties of violas follows:

Chantreyland: The apricot flowers are dwarfed (plate 6, *C*) frequently with pale, yellow, ruffled upper and lateral petals.

Papilio: White streaks appear on upper and lateral pale-blue petals.

Papilio Violet Lilac: White streaks appear on upper and lateral pale-blue petals; the margins are frequently rolled inward.

Heavenly Blue: The blue petals become blanched with a few pale-blue blotches and streaks (plate 6, *B*).

Purple King: Pale-purple blotches and streaks develop in the normal deep-blue color of the flower.

Recovery of Virus. Common-cucumber-mosaic virus was recovered and transferred to cucumbers, to *Nicotiana glutinosa,* or to Turkish tobacco (*N. tabacum*) by mechanical inoculation from only eight varieties of pansies and violas showing breaking as follows: Lord Beaconsfield, Prince Henry, Lake of Thun (Ullswater), Madam Steele, Chantreyland, Papilio, Papilio Violet Lilac, and Purple King. The virus was recovered from Tufted "Pansy Violet", Prince Henry, and Victoria, though they showed no symptoms. Repeated failures were obtained in attempts to recover the virus from the following varieties of pansies which showed breaking: Adonis, Emperor William, Geneva Giant mixed, King of the Blacks, Blue Perfection, and Heavenly Blue.

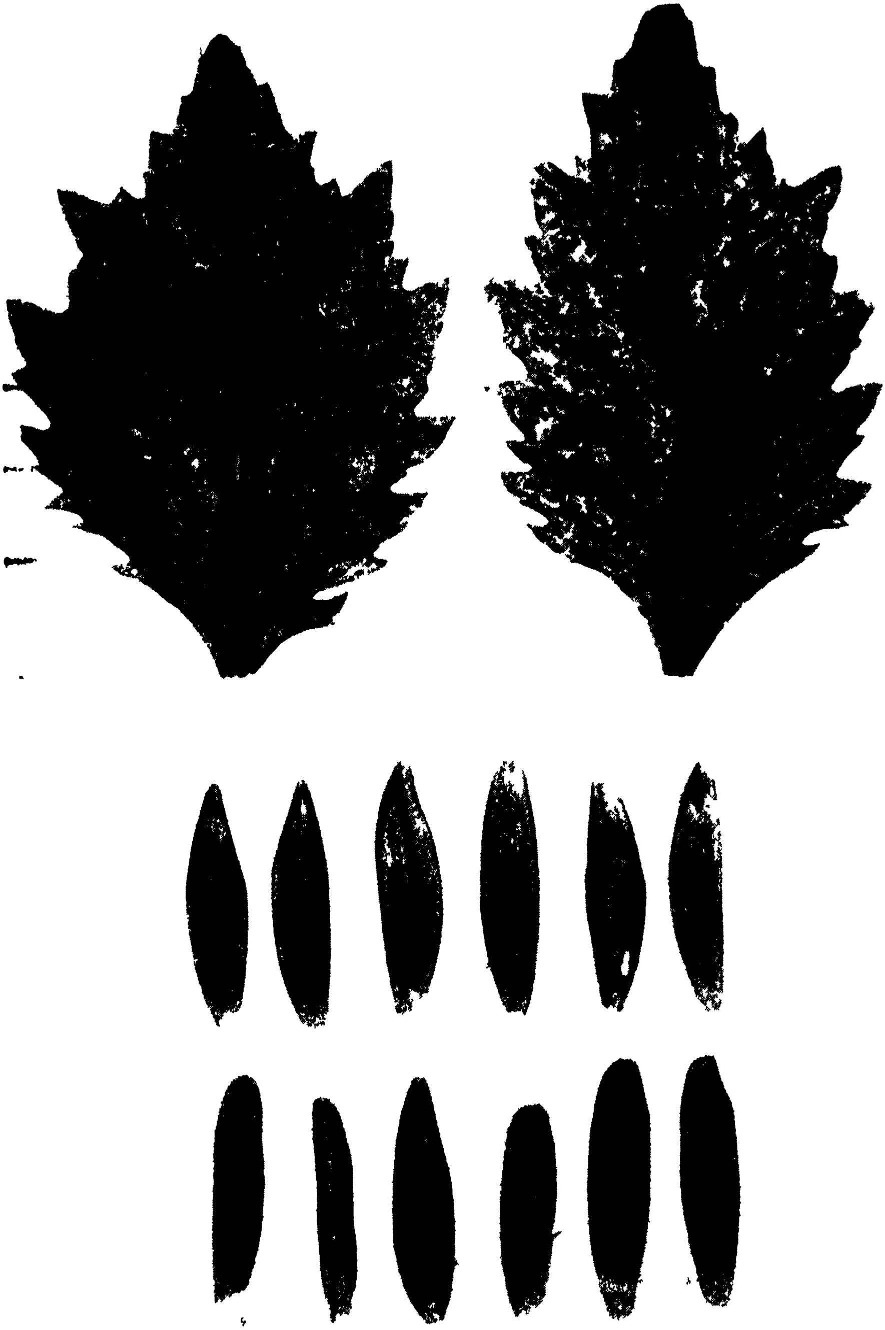

Fig. 1.—China aster (*Callistephus chinensis*) infected with the virus of spotted wilt: upper, leaves showing necrosis; lower, petals showing breaking in color.

VIRUSES CAUSING BREAKING IN OTHER FLOWERS

As reported by Tompkins (1939), the author demonstrated that the false cabbage or turnip aphid, *Lipaphis pseudobrassicae* (Davis) is the vector of mild and severe mosaic viruses in annual stock, or gilliflower (*Matthiola incana* var. *annua*) in nature. Experimental transmission was obtained in greenhouse tests with this species of aphid, and also with the cabbage aphid, *Brevicoryne brassicae* Linneaus, and the green peach aphid, *Myzus persicae* (Sulzer).

The false cabbage or turnip aphid was used in tests to determine whether the mild-mosaic virus induces breaking in violas. Noninfective, mature, wingless aphids reared on mild-mosaic stock plants were transferred in lots of 20 aphids to each of 5 Blue Perfection, 5 Ruby, 5 White Eye violas, and 5 stock plants. Breaking occurred only in the stock plants.

Since the lily aphid, *Myzus circumflexus* (Buckton) is a vector of the celery-calico virus, tests were made with this aphid to determine whether this virus causes breaking in annual stock plants. Mature, wingless aphids reared on Papilio violas infected with celery-calico virus were transferred in lots of 20 aphids to each of 10 healthy stock plants, but no breaking occurred.

The virus extract prepared from stock plants infected with mild mosaic was mechanically inoculated into 5 Blue Perfection violas, 5 Papilio violas, and 5 stock plants. Breaking occurred only in the stock plants.

The virus extract from Papilio violas infected with celery calico was mechanically inoculated in 5 Papilio violas and 5 stock plants. Breaking occurred in the violas but not in stock plants.

Tomato-spotted-wilt virus induced breaking of China asters (*Callistephus chinensis*) (fig. 1), but this virus failed (in mechanical-inoculation experiments) to cause breaking in Papilio violas grown from seeds and Radio violas from cuttings. Celery-calico virus from infected violas failed to induce breaking in China asters.

APHID TRANSMISSION OF VIRUSES

The other paper in this issue (Essig, 1947) discusses the characters, distribution, and food plants of aphid species which have been reported on pansies and violas under natural conditions. Five of these that occur in California were tested to determine whether they transmit celery-calico virus to pansies and violas, and three were tested with western-cucumber-mosaic virus. Some aphids that breed on celery but have not been reported on pansies and violas under natural conditions were also tested for transmission of celery-calico virus.

Vectors of Celery-Calico Virus. The following species of aphids, which have been reported to occur on pansies and violas, or tufted pansies, under natural conditions, were demonstrated to be vectors of the celery-calico virus from infected pansies, violas, and celery to pansies and violas:

Cotton or melon aphid, *Aphis gossypii* Glover
Lily aphid, *Myzus circumflexus* (Buckton)
Foxglove aphid, *Myzus solani* (Kaltenbach)
Violet aphid, *Micromyzus violae* (Pergande)

The celery-calico virus was transmitted from celery to pansies and violas by the following aphid species, which breed on celery under natural conditions (see Essig, 1938):

Celery leaf aphid, *Aphis apigraveolens* Essig
Celery aphid, *Aphis apii* Theobald (*A. helianthi* Monell?)[4]
Rusty-banded aphid, *Aphis ferruginea-striata* Essig
Erigeron root aphid, *Aphis middletonii* Thomas
Yellow willow aphid, *Cavariella capreae* (Fabricius)
Green peach aphid, *Myzus persicae* (Sulzer)
Honeysuckle aphid, *Rhopalosiphum conii* (Davidson)

Vectors of Western-Cucumber-Mosaic Virus. The following species of aphids are vectors of the western-cucumber-mosaic virus:

Cotton or melon aphid, *Aphis gossypii* Glover
Bean or dock aphid, *Aphis rumicis* Linnaeus
Green peach aphid, *Myzus persicae* (Sulzer)

INSECTS WHICH FAILED TO TRANSMIT CELERY-CALICO VIRUS

Severin (1943) reported breaking in color of flower petals of phlox (*Phlox drummondii*) induced by the California aster-yellows virus transmitted by the short-winged aster leafhopper, *Macrosteles divisus* (Uhler) and the long-winged aster leafhopper, a biological race of the same species (Severin, 1940). Tests were made to determine whether either of these could transmit the celery-calico virus. Sixteen lots of 25 or 35 noninfective males of each vector were fed for 2 days on Papilio violas infected with celery-calico virus, and then each lot was transferred to a healthy Papilio viola grown from seeds. No breaking occurred in the 16 plants exposed to the leafhoppers.

CONTROL

Field investigations were carried on for the past twelve years to determine the source of the celery-calico-virus infection in pansies and violas and the spread of this disease in home gardens. Many retail nurseries were visited in the East Bay districts, and it soon became evident that nurserymen who do not spray to control aphids on pansies and violas disseminate the disease to home gardens by selling infected plants. In one retail nursery all of the flats containing Papilio violas showed breaking and the plants were covered with aphids. When the flats were removed from the racks and the violas were allowed to dry in the nursery, the infective aphids spread to pansies, violas, other susceptible ornamental host plants, and weeds. Home gardeners should avoid purchasing pansies and violas showing breaking and aphid infestation.

One nurseryman made cuttings from the perennial Radio viola. An examination of the cuttings in the greenhouse showed breaking in a high percentage. This nurseryman rogued all of the diseased Radio violas grown out of doors and sprayed weekly to control the aphids. The following year not a single cutting showed breaking in the flowers in the greenhouse.

The recommendation for controlling celery calico and western cucumber mosaic in pansies and violas is to spray these plants and susceptible economic plants weekly until the vegetation on the foothills becomes dry, since many species of aphids fly from the foothills. The spray should be applied to the lower surface of the leaves, those which spread on the ground being raised with the fingers. During the summer, spraying can be discontinued, since aphids do not multiply during hot weather. All pansies and violas showing breaking should be rogued and burned. Avoid purchasing pansies and violas showing breaking and aphid infestation.

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[4] According to E. O. Essig (personal interview), *Aphis apii* may be identical with *A. helianthi.*

The insecticide which gave satisfactory results to control aphids was three teaspoons of Black Leaf 40 to a gallon of water, with Volck or soap used as an adherent.

SUMMARY

The symptoms induced by three cucumber-mosaic viruses which cause breaking in color of flower petals of varieties of pansies (*Viola tricolor* var. *hortensis*) and violas, or tufted pansies (*V. cornuta*) are described in this paper. Of these cucumber-mosaic viruses, celery calico and western cucumber mosaic occur in California, but common cucumber mosaic is not known to occur here. Celery calico is common in the coastal fog belt and also occurs in the hot interior regions of California. Western cucumber mosaic occurs only in the interior regions of California and not in the coastal fog belt.

The following species of aphids which have been reported to occur on pansies and violas under natural conditions were demonstrated to be vectors of the celery-calico virus:

Cotton or melon aphid, *Aphis gossypii* Glover
Lily aphid, *Myzus circumflexus* (Buckton)
Foxglove aphid, *Myzus solani* (Kaltenbach)
Violet aphid, *Micromyzus violae* (Pergande)

Infections of pansies and violas with celery-calico virus were obtained with the following aphid species, which breed on celery under natural conditions and also transmit the celery-calico virus to this host plant:

Celery leaf aphid, *Aphis graveolens* Essig
Celery aphid, *Aphis apii* Theobald (*A. helianthi* Monell?)
Rusty-banded aphid, *Aphis ferruginea-striata* Essig
Erigeron root aphid, *Aphis middletonii* Thomas
Yellow willow aphid, *Cavariella capreae* (Fabricius)
Green peach aphid, *Myzus persicae* (Sulzer)
Honeysuckle aphid, *Rhopalosiphum conii* (Davidson)

The following species of aphids have been proved to be vectors of the western-cucumber-mosaic virus:

Cotton or melon aphid, *Aphis gossypii* Glover
Bean or dock aphid, *Aphis rumicis* Linnaeus
Green peach aphid, *Myzus persicae* (Sulzer)

Aphids transmitting the viruses of celery calico and western cucumber mosaic to pansies and violas can be controlled by using a Black Leaf 40 spray.

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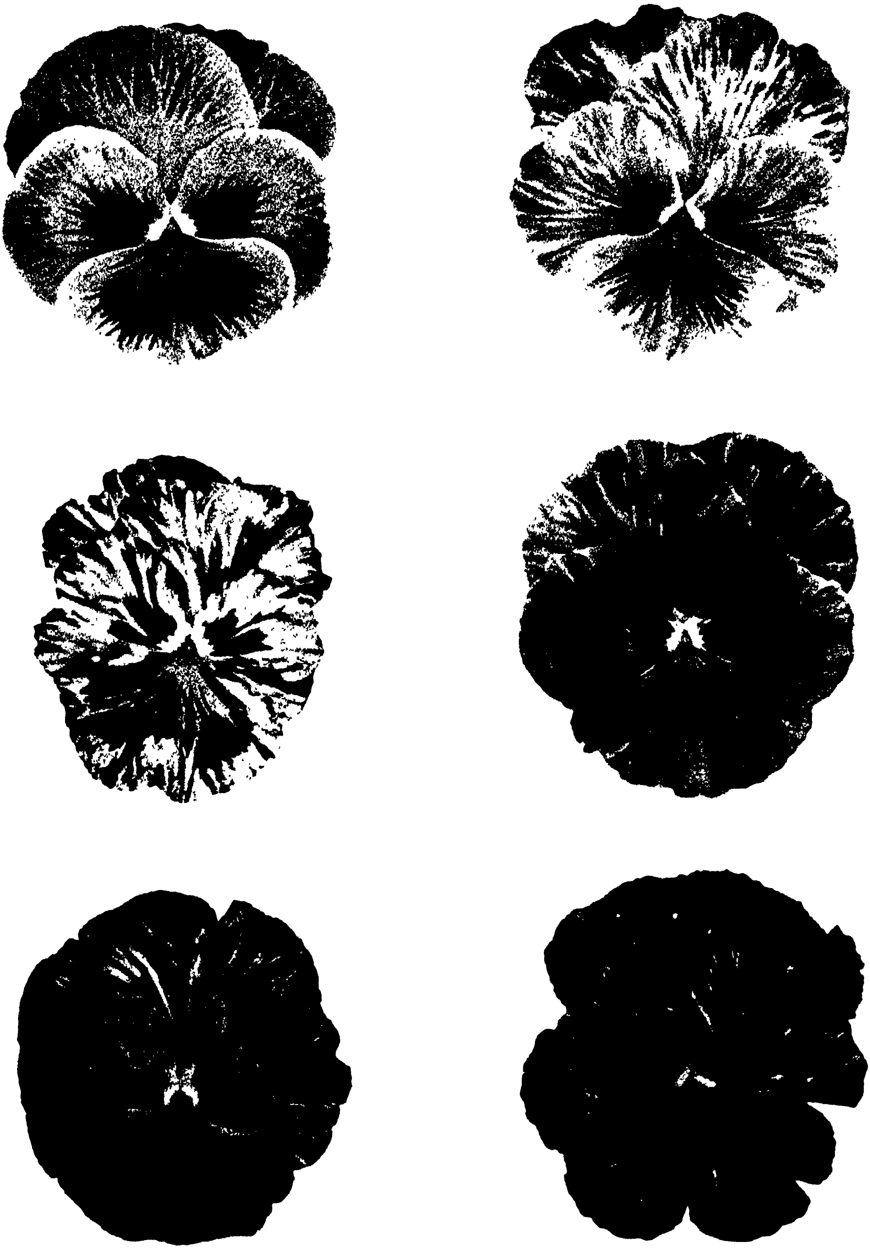

Plate 1. Jumbo pansy (*Viola tricolor* var. *hortensis*) naturally infected with celery-calico virus: top row, Golden Yellow strain, the left showing yellow streaks, the right, white streaks; center row, left, blue and yellow pansy with blue, purple, and yellow bands; center row, right, brown pansy with yellow and brown bands; bottom row, left, purple strain showing pale- and dark-purple streaks; bottom row, right, blue pansy showing white streaks on the petals.

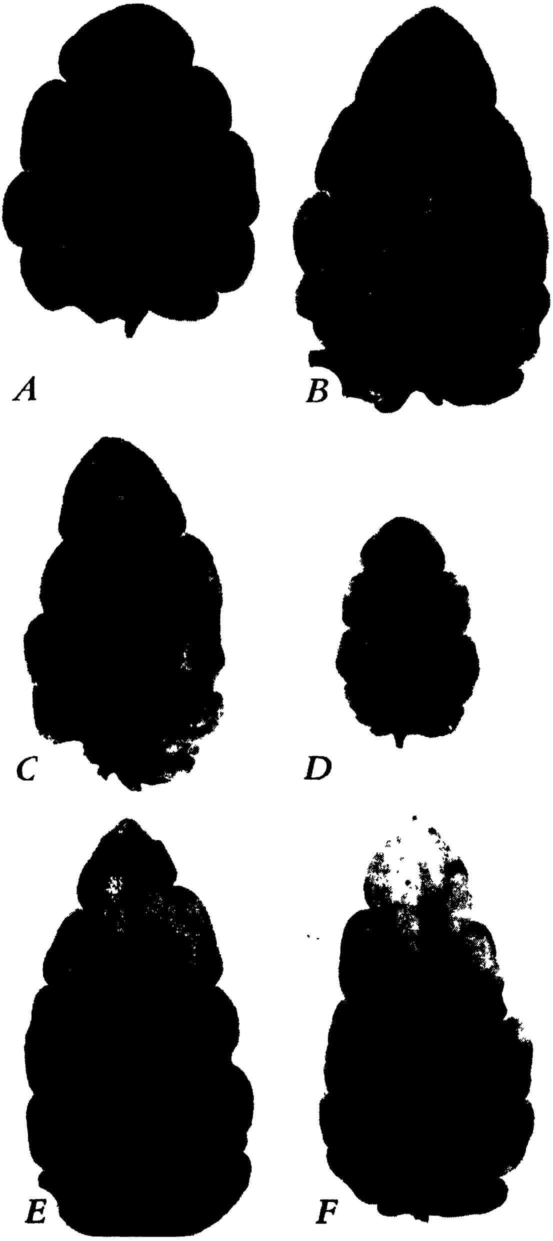

Plate 2. Symptoms of celery calico on the leaves of Papilio viola (*Viola cornuta*): *A*, leaf from healthy check or control plant; *B*, cleared venation on basal half of leaf; *C*, cleared veins and veinlets on entire leaf; *D*, small, chlorotic, circular areas; *E*, yellowing of leaf; *F*, lemon-yellow at tip and margin of leaf.

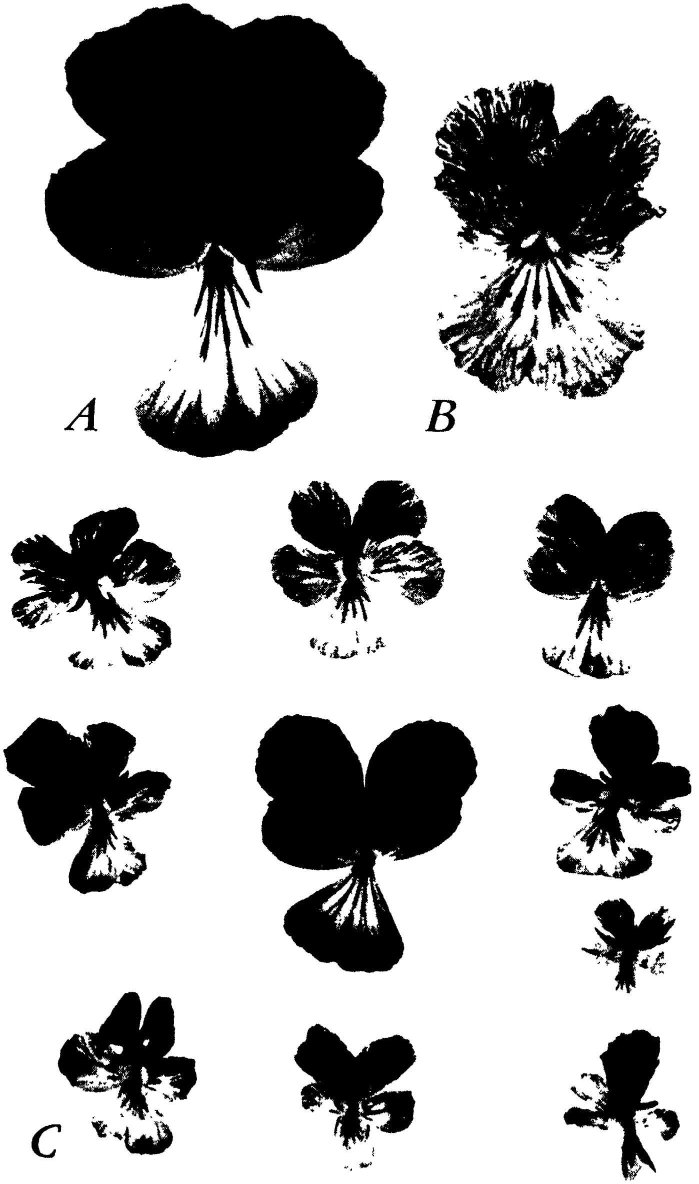

Plate 3. Breaking in color of flower petals of Papilio viola (*Viola cornuta*): *A*, flower from healthy plant; *B*, flower showing white streaks on petals, from a plant inoculated with the virus extract from perennial delphinium naturally infected with celery calico collected at Colma November 6, 1937; *C*, center: normal flower from a healthy plant. Grouped around it are dwarfed flowers showing breaking in the color of the petals, from plants naturally infected with the celery-calico virus. (El Cerrito, May 29, 1935).

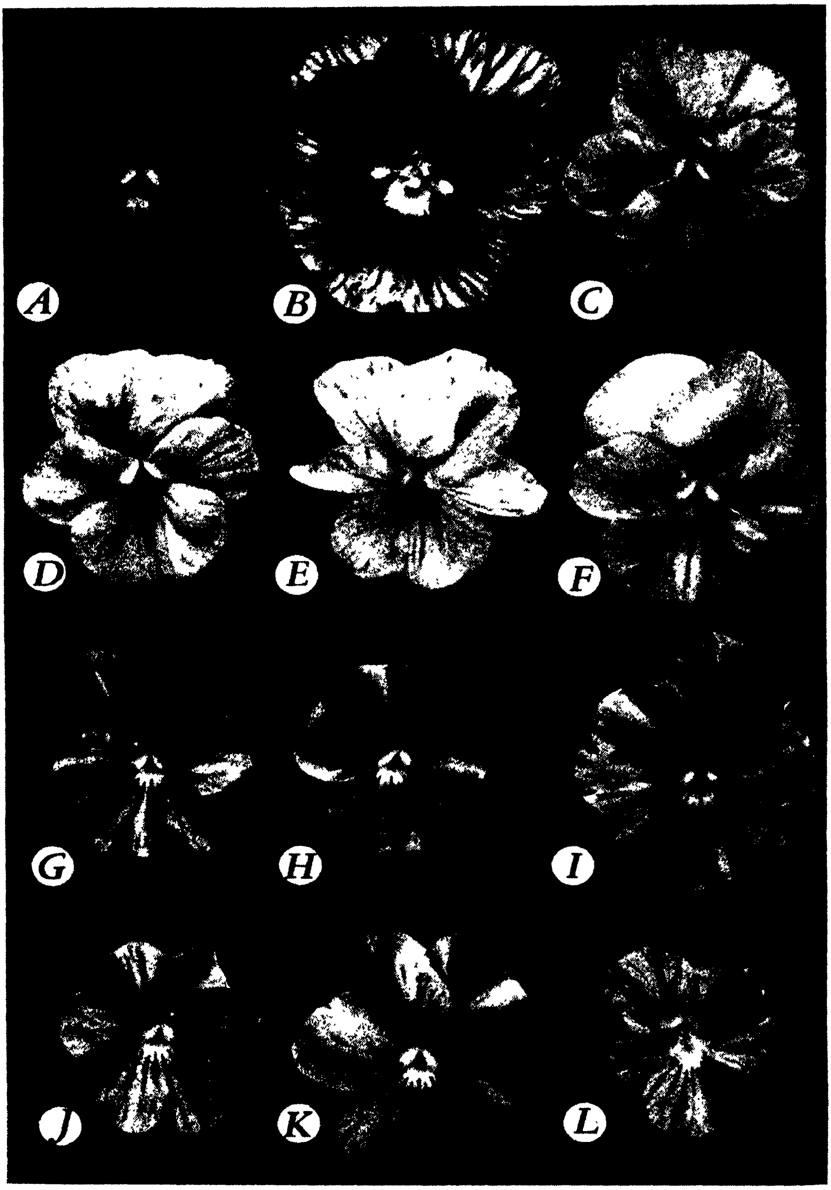

Plate 4. Radio viola (*Viola cornuta*) showing breaking in color of petals from plants naturally infected with celery-calico virus: *A*, flower from healthy plant; *B*, flower showing white streaks; *C, D, E*, successive stages in blanching with few streaks of normal color of flower; *F*, blanched flower showing no streaks (Berkeley, August 23, 1934); *G, H, I, J, K*, blotching and streaking; *L*, dwarfed flower showing white streaks (Berkeley, June 16, 1936).

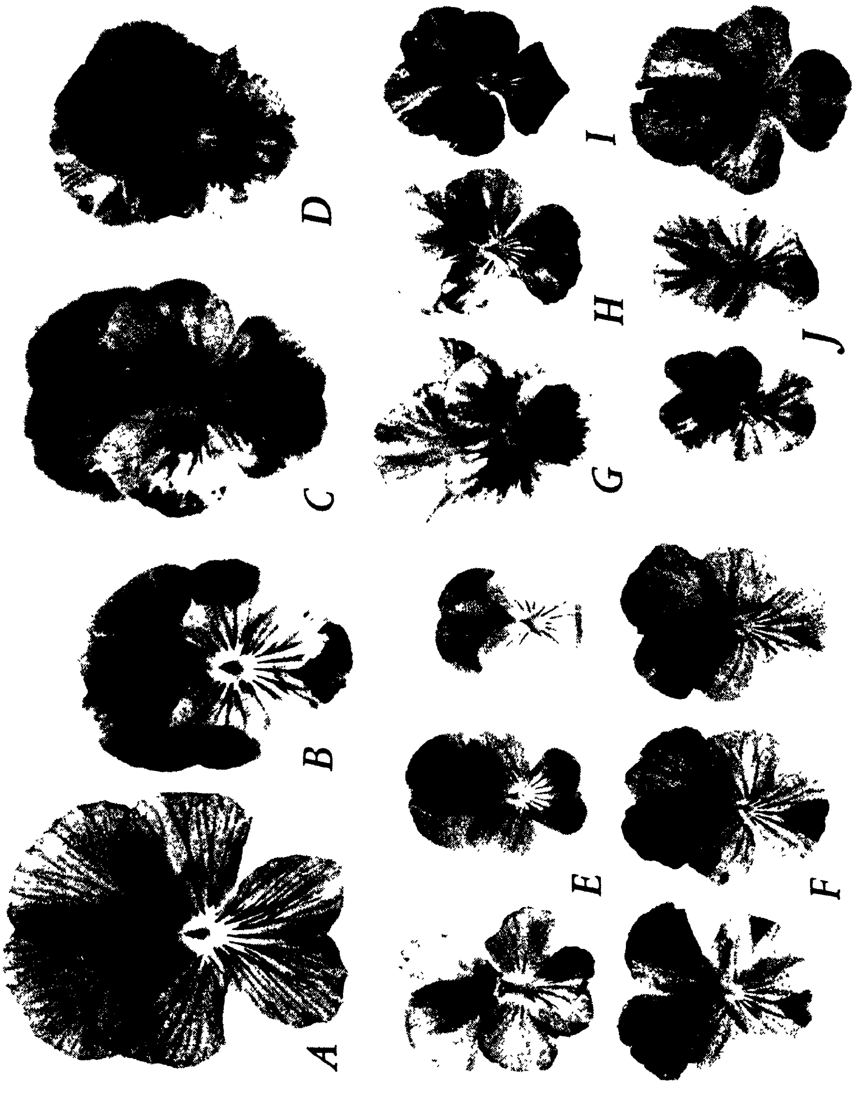

Plate 5. Tufted pansies or violas (*V. cornuta*) showing breaking in color of flower petals induced by western-cucumber-mosaic virus (*B*, *D*, *F*, *G*, *H*, *I*) and celery-calico virus (*E*, *J*). *A*, *B*, Heavenly Blue viola, *A* showing normal flower from healthy plant and *B*, a dwarfed flower from infected plant with normal, deep-blue color on upper petals and deep-blue blotches and pale-blue center on lateral and lowest petals; *C*, Tufted "Pansy Violet" showing normal flower from check or control plant; *D*, dwarfed flower with irregular margin showing numerous white streaks on petals; *E*, Lilac viola, flowers from plant inoculated with celery-calico virus; *F*, blossoms from plant inoculated with western-cucumber-mosaic virus showing dwarfing and stages of blanching of petals; *G*, Tufted "Pansy Violet" showing white streak and early blanching; *H*, Lake of Thun, or Ullswater pansy, dwarfed flower showing blanching; *I*, normal flower from check or control plant; *J* (lower row), Lilac viola, showing blanching and white streaks.

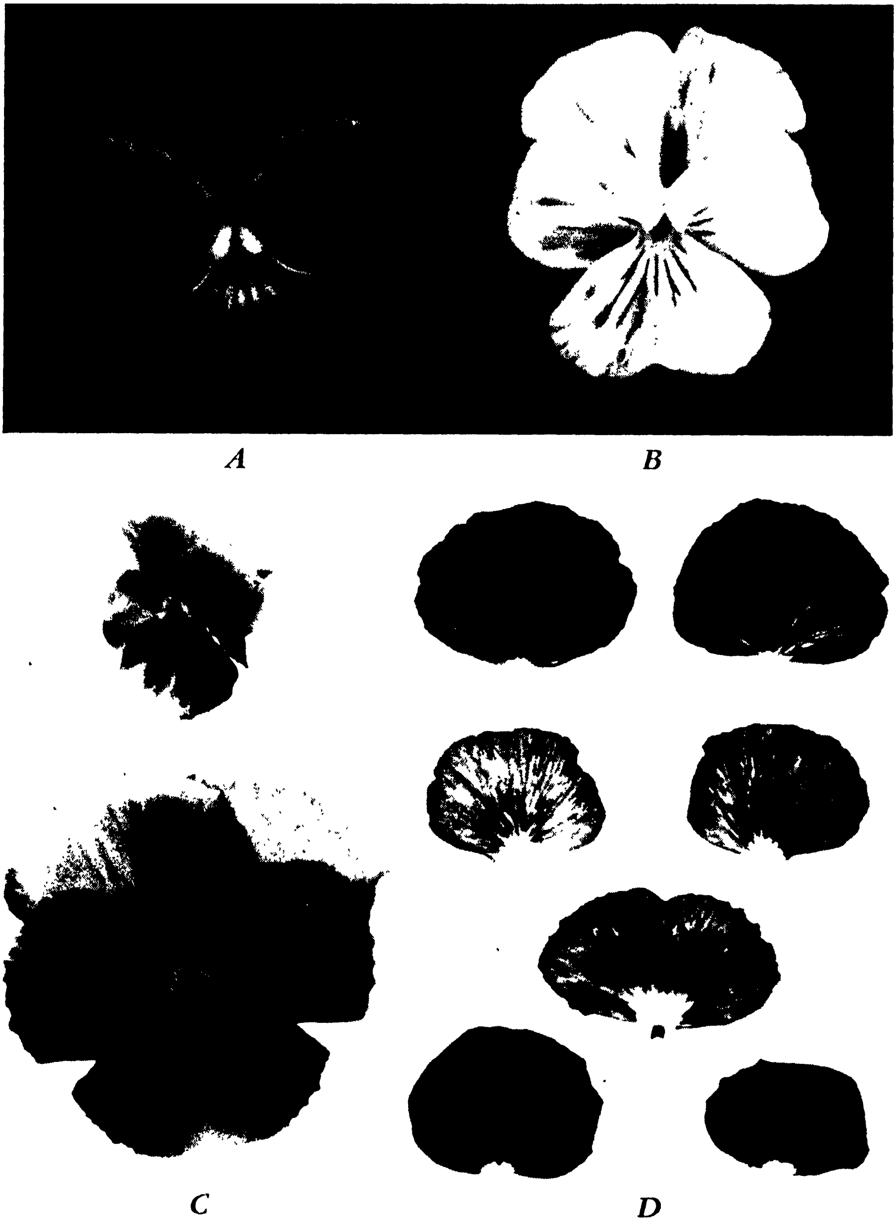

Plate 6. Pansy (*Viola tricolor* var. *hortensis*) and tufted pansy, or viola (*V. cornuta*) showing breaking in color of flower petals induced by common-cucumber-mosaic virus: *A*, *B*, Heavenly Blue viola, *A* showing normal flower from healthy check or control; and *B*, blanched petals with a few pale, blue blotches and streaks; *C*, Chantreyland viola, upper, dwarfed flower showing white blotches; lower, normal flower from healthy plant; *D*, Lake of Thun, or Ullswater, pansy, upper five petals showing short white streaks; lower, two petals from check or control plant.

APHIDS FEEDING ON VIOLACEOUS PLANTS IN CALIFORNIA

E. O. ESSIG

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APHIDS FEEDING ON VIOLACEOUS PLANTS IN CALIFORNIA[1]

E. O. ESSIG[2]

PANSIES (*Viola tricolor* var. *hortensis*) and violas, or tufted pansies (*V. cornuta*) in California are subject to two virus diseases, celery calico and western cucumber mosaic. These diseases are treated in a companion article (Severin, 1947).[3] The vectors are aphids, or plant lice. Celery calico was first investigated (Severin and Freitag, 1938) as a disease of celery. There are no less than ten aphid species feeding on celery in California. Other species which ordinarily do not feed and propagate on this host may also transmit the celery-calico virus under experimental conditions.

The only species apparently restricted to violaceous plants in this state and in other parts of North America is the common violet aphid, *Micromyzus violae* (Pergande). Nine additional species have been recorded as feeding on *Viola* spp. (Patch, 1938) in various parts of the world.

The purpose of this paper is to furnish those interested in the virus diseases of plants, particularly of ornamental violas and pansies, pertinent information concerning the aphid vectors of these diseases. Certain of these aphids have previously been discussed in relation to celery calico as a disease of celery in California (Essig, 1938*a*); and therefore the treatment of these particular aphid species excludes detailed descriptions and emphasizes more particularly synonymy, host plants, distribution, and bibliography.

It is expected that the illustrations may enable those interested in this problem to identify the aphids responsible for the dissemination of the celery-calico and western-cucumber-mosaic viruses.

THE PEA APHID

Macrosiphum pisi (Kaltenbach)
Aphis pisi Kaltenbach (1843, p. 23–24)
Aphis ulmariae Schrank (1801, p. 116–17)
Aphis onobrychis B. de Fonscolombe (1841, p. 61)
Adactynus pisi (Kaltenbach) (Rafinesque 1819, p. 18)
Siphonophora corydalis Oestlund (1886, p. 25–26)
Nectarophora destructor Johnson (1900, p. 58–59)
Macrosiphum trifolii Pergande (1904, p. 21–23, fig. 4)
Illinoia pisi Wilson (1910 *b*, p. 318)
Acyrthosiphon onobrychis (B. de Fonscolombe)

The pea aphid is one of the largest and most conspicuous economic aphids. Its all-green color and its propensities for leguminous plants make it easily recognized. In common with many other aphids, this species drops to the ground when the host plants are disturbed, a habit that often enables the individuals to conceal themselves under clods of earth and debris and thus escape

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[1] Received for publication April 24, 1947.

[2] Professor of Entomology and Entomologist in the Experiment Station.

[3] See "Pertinent Literature," at the end of this paper, for complete citations, referred to in text by author and date.

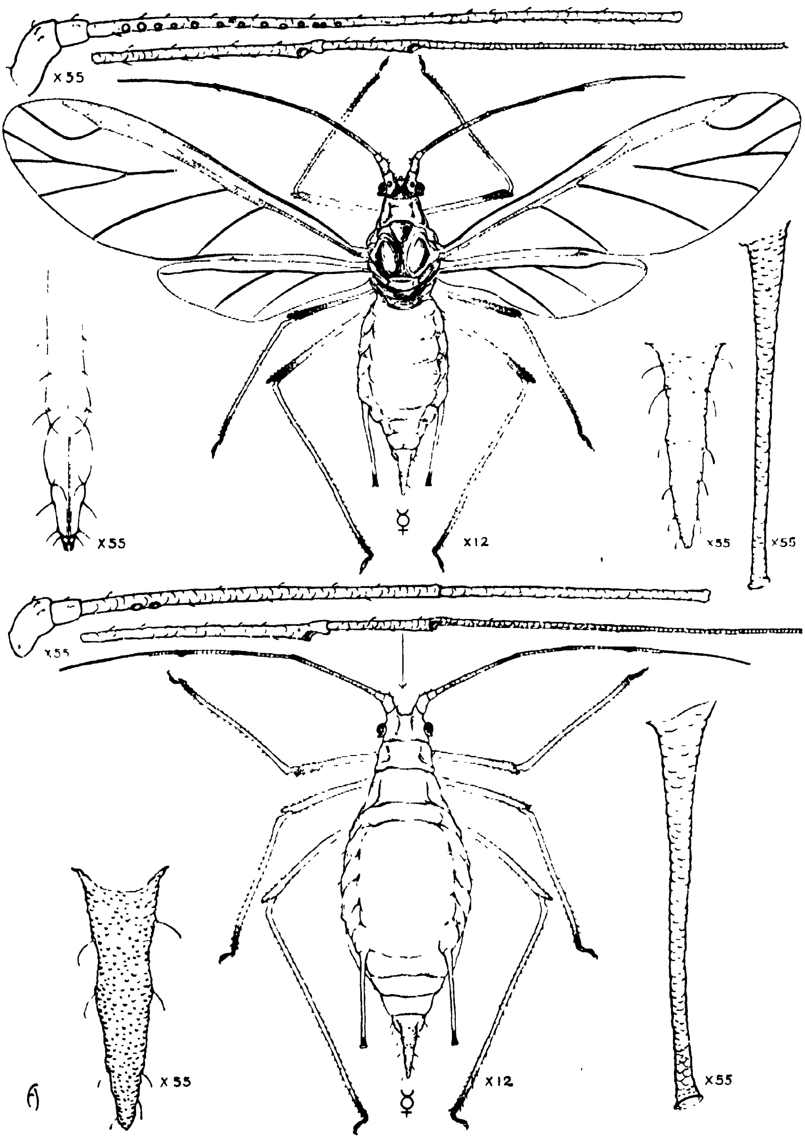

Fig. 1.—The pea aphid, *Macrosiphum pisi* (Kaltenbach): alate and apterous females with enlargements of the antennae, caudas, cornicles, and rostrum. (Enlargements indicated. Drawings by Frieda Abernathy).

insecticides. Among the many references to this destructive pest, the most important are Pergande (1904, p. 21–23), Davis (1915), and Soliman (1927, p. 132–33.

C. D. Harrington (1941, p. 461–66) has shown that a number of more or less distinct strains of the pea aphid occur in various parts of the country. Members of these strains may vary considerably in size, reproductive capacities, rate of development, destructiveness to the host plants, and resistance to insecticides. The adult apterous parthenogenetic females may attain a length of 2.5 to 3.5 mm.

In California the pea aphid is not known to produce sexual forms, although the latter may occur at higher altitudes of the Sierra Nevada. The aphid continues throughout the entire year as parthenogenetic apterous and alate females. During the winter, adults hibernate or may continue to reproduce young on perennial hosts. During the spring, summer, and fall, many generations are born and very large populations may occur in any month of the year. The greatest numbers appear in early spring and continue abundant until midsummer.

Nomenclature. Some authors believe that Schrank's name, *ulmariae*, should supersede *pisi* as the specific name. But the original description by Schrank seems to indicate that *ulmariae* is not synonymous with *pisi* Kalt. His statements that the antennae are shorter than half the length of the body and that the cornicles (*Seftspizen*) are longer than the very short cauda (*"Schwänzchen am After"*) are certainly not characteristic of *pisi*, in which the antennae are as long as the body or longer and the cauda is conspicuously well developed.

Aphis onobrychis B. de Fonscolombe may well have precedence over all other specific names. *Macrosiphum onobrychis* B. de Fonsc. is the name being used in the publications of the Imperial Institute, London, England. But the combination *Macrosiphum pisi* (Kalt.) has been used so extensively in entomological literature in North America that changing it seems unwise.

The genus *Adactynus* was erected by Rafinesque (1817, p. 18).

The genus *Illinoïa* was erected by Wilson (1910*b*, p. 318) for this species but has not been generally adopted. It has precedence over *Metopeurum* Mordvilko (1914, p. 56, 67) and *Acyrthosiphon* Mordvilko (1914, p. 55, 62).

The generic name *Acyrthosiphon* has been extensively used for the pea aphid in Europe and by some American authors. The long array of names now being given to this insect indicates the need of greater attention to these matters by national and international commissions of nomenclature.

Distribution. The pea aphid has been reported from all of the continents and is widely distributed throughout the world wherever food plants are available.

It occurs in every state in this country and in all the provinces of Canada. Its powers of flight are great and it often swarms in tremendous numbers and is carried long distances by air currents during the night as well as the day, especially in warm summer weather.

Host Plants. Although the preferred host plants are confined to the single family, Leguminosae, the members of this plant group are so extremely numerous that they cover much of the habitable areas of the world. Many of the host plants produce valuable crops for man and his domestic animals. The injuries caused by the pea aphid may result in great loss to these crops and even complete failure. Such injuries may result from the aphid's feeding on and actually stunting or killing the plant or from the aphid's inoculating the hosts with virus diseases which may weaken or completely destroy the plants. Plants, other than legumes, are frequently listed as hosts of the pea aphid. The adults or young may feed on some of those plants and thus transmit a virus disease. However, rearing of the young is usually impossible on the nonleguminous hosts, and on many of these plants the insect may be only a casual visitor.

The pea aphid may be able to feed intermittently on violets and pansies as well as upon many other plants. The feeding in itself is not injurious; the damage the aphid does to these hosts is to infect them with the virus disease which may destroy them.

The pea aphid was reported from *Viola* by Swain (1919, p. 176) and Patch (1938, p. 181). As already pointed out, however, this aphid is a strong traveler, both on foot and on the wing, and is a casual visitor on many plants upon which it may not actually feed.

A complete list of the known host plants follows:

Alhagi maurorum
Capsella bursa-pastoris
Celastrus scandens
Clianthus puniceus
Colutea arborescens
Cytisus scoparius
Genista tinctoria
Glycine soja
Igatium janium (*Ignatia?*)
Lathyrus hirsutus
Lathyrus latifolius (*L. angustifolius*)
Lathyrus maritimus
Lathyrus odoratus
Lathyrus pisiformis
Lathyrus pratensis
Lathyrus sativus
Lathyrus sylvestris
Lespedeza cytisoides
Lotus corniculatus
Lotus oblongifolius
Lupinus spp.
Medicago falcata
Medicago hispida
Medicago lupulina
Medicago sativa
Melilotus alba
Melilotus indica
Melilotus officinalis
Onobrychis viciaefolia (*O. sativa*)
Ononis repens
Peganum harmala
Phaseolus vulgaris
Pisum sativum
Pisum sativum var. *arvense* (*P. arvense*)
Psoralea macrostachya
Robinia pseudoacacia
Trifolium agrarium
Trifolium alexandrinum
Trifolium arvense
Trifolium filiforme
Trifolium hybridum
Trifolium incarnatum
Trifolium pratense
Trifolium procumbens
Trifolium repens
Trifolium scabrum
Trigonella foenum-graecum
Vicia americana
Vicia amoena
Vicia atropurpurea
Vicia angustifolia
Vicia cracca
Vicia faba
Vicia gigantea
Vicia ludoviciana
Vicia sativa
Vicia sepium
Vicia villosa
Vigna sinensis
Viola spp., (casual?)
Zizia aurea

THE COTTON OR MELON APHID

Aphis gossypii Glover

The cotton or melon aphid, *Aphis gossypii* Glover, is a prolific, widely distributed, and omnivorous species, which is responsible for transmitting many mosaic and virus diseases of plants. It is very common and abundant throughout much of California and is widely distributed in the United States. It also occurs abundantly in the tropics.

This aphid is fully discussed in a previous paper (Essig, 1938*a*, p. 468–70). It is a small species, often blackish or very dark olive green in color, but frequently pale white, yellow, orange, or green, on different host plants.

Distribution. The cotton aphid has been recorded from the following countries:

Africa: Algeria, Anglo-Egyptian Sudan, Belgian Congo, British Togoland, Portuguese East Africa, Egypt, Eritrea, French West Africa, Italian Somaliland, Morocco, Nigeria, Nyasaland, Rhodesia, South Africa, Tanganyika Territory, and Uganda.

Asia: Armenia, Asiatic Russia, Astrakhan, Ceylon, China, India, Japan, Malaya, Palestine, Pescodores Islands, Syria, Taiwan, Transcaucasia, and Turkestan.

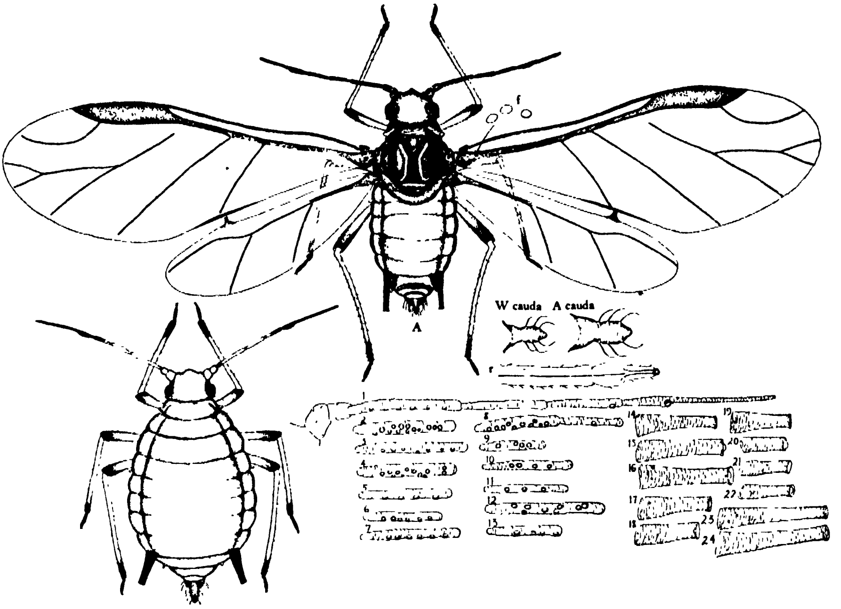

Fig. 2.—The cotton or melon aphid, *Aphis gossypii* Glover: *A*, winged viviparous female; *B*, apterous viviparous female; *W cauda*, cauda of winged female: *A cauda*, cauda of apterous female; *f*, fenestras; *r*, rostrum, *1*, antenna of winged female on cotton, California; *2–13*, antennal segment III of winged female drawn to scale to show variation in number of secondary sensoria and length. (All greatly enlarged.)

Europe: Belgium, Crimea, Cyprus, Denmark, France, Germany, Great Britain, Italy, Netherlands, U.S.S.R., Sweden, Switzerland.

Central America: Canal Zone, Panama.

North America: Bermuda, Canada, Mexico, United States, West Indies (Cuba, Jamaica, Puerto Rico).

South America: Argentina, Brazil, British Guiana, Chile, Dutch Guiana, Paraguay, Peru, Trinidad Island.

Oceania and the Pacific: Australia, Dutch East Indies, Fiji, Guam, Hawaii, Philippine Islands, Samoa.

In California it is found abundantly throughout the state except in high altitudes above 6,000 feet.

Host Plants. The cotton aphid is an omnivorous feeder, being specially destructive to cacao, citrus, cotton, cucumbers, melons, squashes, hibiscus, lilies, and many other plants. A complete list of host plants follows:

Abutilon theophrastii
Acalypha boemeroides
Acalypha virginica
Acanthopanax trifoliatus
 (*A. aculeatus*)
Acanthus ilicifolius
Ageratum conyzoides
Allium cepa
Althaea nudiflora
Althaea officinalis
Althaea rosea
Amaranthus viridis
Ambrosia sp.
Annona muricata
Anthemis sp.
Antirrhinum spp.
Antigonon leptopus
Aphelandra pectinata
Apium graveolens
Aquilegia vulgaris
Arabidopsis thaliana
Arabis drummondii
Arctium lappa
Ardisia esculenta
Aristolochia indica
Asclepias mexicana
Asclepias speciosa
Asclepias verticillata
Asclepias vestita
Asparagus officinalis
Atriplex sp.
Avena fatua
Bauhinia variegata
Begonia semperflorens
Benincasa hispida
Beta vulgaris
Bidens frondosa
Bidens pilosa
Blumea balsamifera
Boerhaavia plumbaginea
 (*B. viscosa*)
Borago officinalis
Bougainvillea spectabilis
Brassica (all species)
Breynia officinalis
Brunnera macrophylla
Buddleia officinalis
Bursera simaruba
Caladium sp.
Callicarpa dichotoma
 (*C. purpurea*)
Callicarpa formosana
Callistephus chinensis
Calophyllum inophyllum
Canna sp.
Capsella bursa-pastoris
Capsicum frutescens
 (*C. annuum*)
Capsicum dulce
Cassia tora
Casuarina equisetifolia
Catalpa sp.
Ceiba pentandra
 (*Eriodendron anfractuosum*)
Cereus sp.
Cestrum diurnum
Chaenomeles japonica
 (*Pyrus japonica*)
Chenopodium album
Chenopodium anthelminticum
Chrysanthemum frutescens
Chrysanthemum morifolium
 (*C. hortorum*,
 C. sinensis)
Chrysobalanus oblongifolius
Cineraria sp. (*Senecio*?)
Cirsium arvense
Citrullus vulgaris
Citrus aurantium
Citrus limonia
Clerodendrum thomsoniae
Clerodendrum trichotomum
Clitoria sp.
Coccolobis flendana
Coccolobis floridana
 (*C. laurifolia*)
Colocasia antiquorum
Colocasia esculenta
Colocasia kotoensis
Combretum apiculatum
Commelina diffusa
Commelina persicariaefolia
 (*C. benghalensis*)
Cordia myxa
Cornus mas
Cosmia sp. (*Cosmos* sp.)
Cosmos caudatus
Crataegus spp.
Crocus dosdell
Crotalaria anagyroides
Crotolaria mucronata
 (*C. striata*)
Cryptostegia grandiflora
Cucumis citrullus
Cucumis melo
Cucumis sativus
Cucurbita maxima
Cucurbita meloriticulatrus(?)
Cucurbita moschata
Cucurbita pepo
Cuphea micropetala
Cyclamen europaeum
Cyclamen indicum
Cynometra ramiflora
 (*C. cauliflora*)
Cyphomandra betacea
Datura stramonium
Delphinium sp.
Dentella repens
Dianthus sp.
Dichrocephala latifolia
Diervilla sp.
Diodia teres
Dipcadi serotina
Dodartia sp.
Dolichos lablab
Duranta repens
 (*D. plumieri*)
Echinocystis fabacea
Echinocystis lobata
Echeveria spp.
Eclipta erecta
Elephantopus scaber
 (*E. mollis*)
Erechtites praealta
Erechtites hieracifolia
Erechtites valerianaefolia
Eriobotrya deflexa
Eriobotrya japonica
Erodium cicutarium
Eucalyptus cladocalyx
 (*E. corynocalyx*)
Eucalyptus globulus
Eugenia aquea
Euphorbia pilulifera
Ficus benjamina
Ficus capensis
Ficus elastica
Flemingia congesta
Forsythia viridissima
Fragaria chiloensis
Fuchsia spp.
Galinsoga sp.
Galium circaezans
Gardenia jasminoides
 (*G. florida*)
Gladiolus haltes
Glycine max (*G. soja*)

Gnaphalium japonicum
Gnaphalium spathulatum
Gossypium herbaceum
Gossypium hirsutum
Helenium autumnale
Helianthus annuus
Heliotropium indicum
Heptapleurum venulosum
Hibiscus abelmoschus
(*H. moschatus*)
Hibiscus eetveldaenus
Hibiscus esculentus
Hibiscus rosa-sinensis
Hibiscus sabdariffa
(*H. suddariffa*)
Hibiscus syriacus
Hibiscus tiliaceus
Hibiscus trionum
Hoffmannia sp.
Humulus lupulus
Hydrangea sp.
Hydrocotyle prolifera
Impatiens spp.
Ipomoea batatas
Ipomoea pandurata
Ixora chinensis
Lactuca debilis
Lactuca sativa
Lagenaria siceraria
(*L. vulgaris*)
Lantana camara
Lantana montevidensis
(*L. sellowiana*)
Lawsonia inermis
Leonurus sibiricus
Leontodon autumnalis
Lepidium virginicum
Leucas lavandulaefolia
Leucas zeylanica
Lilium candidum
Lilium formosanum
Lilium longiflorum
Lilium regale
Lilium speciosum
Lilium testaceum
Liquidambar formosana
Lonicera spp.
Loranthus sp.
Luffa acutangula
Lycopersicon esculentum
(*Solanum lycopersicum*)
Lysimachia clethroides
Lysimachia stricta
Magnolia sp.
Malpighia sp.
Malus coronaria
(*Pyrus coronaria*)
Malus domestica
(*Pyrus malus*)
Malva alcea
Malva parviflora
Malva rotundifolia
Malva sylvestris
Malvastrum fasciculatum
Mammea americana
Mangifera indica
Medicago sativa
Melastoma candidum
Melilotus alba
Melochia corchorifolia
(*Riedlea corchorifolia*)
Melothria maderaspatana
Mentha spicata
(*M. viridis*)
Mentzelia lindleyi
(*Bartonia aurea*)
Monarda fistulosa
Morinda sp.
Mosla formosana
Nasturtium indicum
Nemesia strumosa
Nepeta hederacea
(*N. glechoma*)
Nicotiana tabacum
Nymphaea spp.
Ochroma pyramidale
(*O. lagopus*)
Ocimum basilicum
Oenothera biennis
Oldenlandia sp.
Orobanche aegyptica
Ourouparia formosana
(*Uncaria* sp.)
Passiflora sp.
Perilla ocymoides
Persea americana
(*P. persea*)
Petasites japonicus
Petunia sp.
Phacelia californica
Phaseolus coccineus
Phaseolus nanus
Phaseolus vulgaris
Philadelphus coronarius
Piper nigrum
Pittosporum rhombifolium
Pittosporum tobira
Plerocarpa mutica
Portulaca americana
Portulaca oleracea
Potentilla sylvestris
Pourthiaea villosa
Pouzolzia indica
Prunus sp.
Psidium guacum
Psidium guajava
Punica granatum
Pyrus communis
Pyrus pyrifolia
(*P. serotina*)
Raphiolepis sp.
Rhamnus purshiana
Rhapis excelsa
Richardia scabra
Rosa sp.
Ruellia difformis
(*Hygrophila difformis*)
Rumex acetosella
Rumex crispus
Sagittaria latifolia
Salix sp.
Salsola kali
Salvia scapiformis
Salvia splendens
Satureia vulgaris
Saxifraga splendens
Scutellaria multiglandulosa
Sedum purpureum
(*S. telephium?*)
Sedum reflexum
Sempervivum sp.
Senecio cruentus
Senecio mikanioides
Senecio vulgaris
Sesamum orientale
(*S. indicum*)
Sida acuta
Solanum aculeatissimum
Solanum melongena
Solanum nigrum
Solanum seaforthianum
Solanum torvum
Solanum tuberosum
Solanum verbascifolium
Sonchus oleraceus
Sophia sp. (*Sisymbrium* sp.? *Pachira* sp.)
Spathodea campanulata
Spergula arvensis
Sphaeranthus sp.
Spinacia oleracea
Stellaria media
Strychnos spinosa
Taraxacum officinale
(*T. dens-leonis*)
Tectona grandis
Tephrosia candida
Tephrosia vogelii
Theobroma cacao
Tighonia grandiflora
Tribulus terrestris

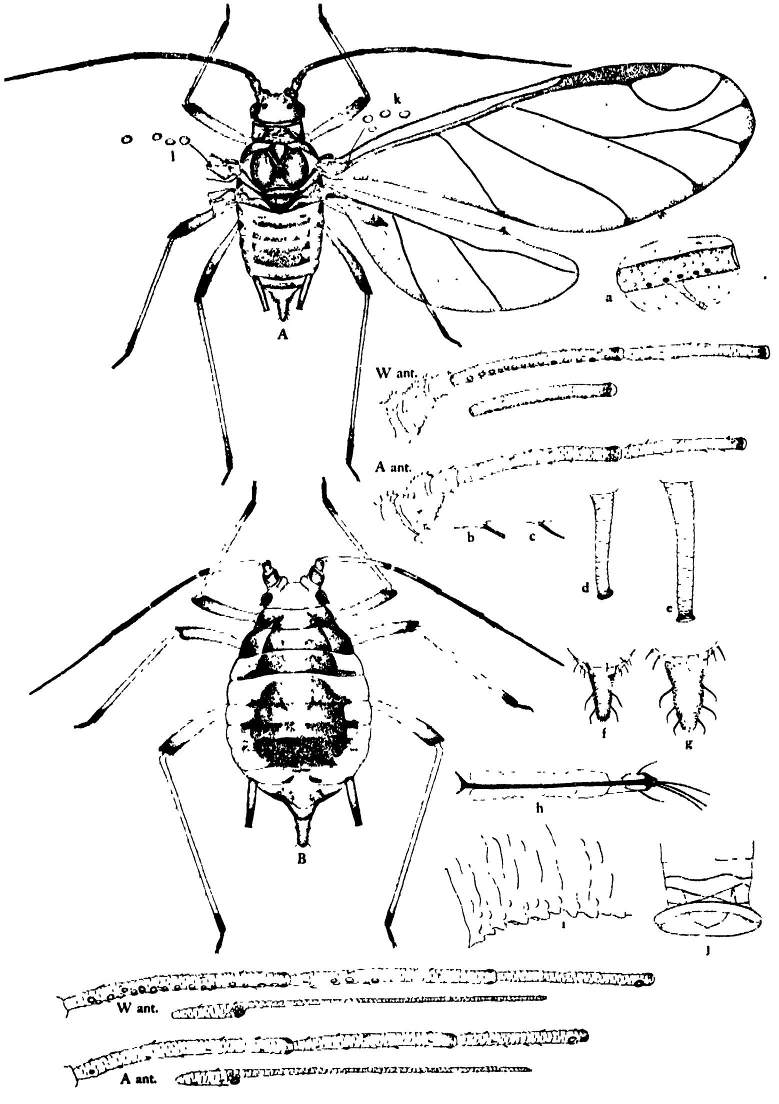

Fig. 3.—The lily aphid, *Myzus circumflexus* (Buckton): *A*, adult winged female: *a*, section of the costal vein showing fenestralike areas; *d*, cornicle; *f*, cauda; *k* and *l*, fenestras near the base of the subcostal vein; *W ant.*, antennae; *B*, adult apterous female: *A ant.*, antennae; *b* and *c*, setas on segments I and III of antenna; *e*, cornicle; *g*, cauda; *h*, rostrum; *i*, basal margin of cornicle; *j*, tip of cornicle. (All greatly enlarged.)

Trifolium pratense
Tulipa sp.
Urena lobata
Valeriana urticaefolia
(*V. valericaefolia*)
Verbena bipinnatifida
Verbena bracteata
Verbena hortensis
(*V. hybrida*)
Veronica chinensis
Veronica officinalis
Veronica serpyllifolia
Vinca major
Vinca minor
Viola odorata
Vitex trifolia
Vitis vinifera
Wedelia sp.
Wisteria floribunda
Wisteria sinensis
Wisteria macrostachya
Wisteria venusta
Zea mays
Zinnia sp.

THE LILY APHID

Myzus circumflexus (Buckton)

Siphonophora circumflexa Buckton (1876, p. 130)

The lily aphid is a common species in greenhouses throughout the country. It also occurs outdoors along the coast of California. It feeds and propagates on a wide variety of plants, on which it sometimes produces dense colonies, although it shows decided preferences for members of the family Liliaceae. The aphids are pale yellow, the alates being partly black, whereas the apterae often have distinct black or dusky patches on the dorsum as shown in figure 3. An extensive treatment of this species is given in a previous paper (Essig, 1938*a*).

Distribution. This aphid has been widely distributed, in commerce, on bulbs and ornamentals. At the present time it is known to occur in the following continents, countries, and states:

Asia: Japan.

Europe: England, Wales, Holland, Russia, Sweden, Switzerland.

North America: Canada; United States, in California, Colorado, Indiana, Illinois, Maine, Michigan, New York, Oregon, Utah, Wisconsin, and possibly in many other states not yet reported.

South America: Argentina.

Pacific: Java, Hawaii.

Host Plants. The lily aphid has been recorded from the following plants:

Adiantum curreatum
(*A. cuneatum?*)
Aesculus californica
Alisma sp.
Alopecurus pratensis
Anemone cylindrica
Antholyza refracta
Anthurium sp.
Apium graveolens
Aquilegia canadensis
Aquilegia canadensis
var. *flavescens*
Arabis sp.
Artemisia dracunculus
Arum sp.
Asparagus plumosus
Aster dracunculoides
Aster dumosus
Aster multiformis
(*A. multiflorus?*)
Avena sp.
Bixa orellana
Brunnera macrophylla
(*Anchusa myosotidiflora*)
Caladium sp.
Calceolaria europaeum
Calendula officinalis
Carduus spp.
Ceanothus sp.
Cerastium viscosum
Cheiranthus cheiri
Chrysanthemum sp.
Crocus sp.
Cyclamen europaeum
Cyclamen indicum
(*C. persicum*)
Cyrtomium sp.
Dahlia sp.
Digitalis sp.
Diospyros sp.
Eichhornia crassipes
Ficus sp.
Freesia refracta
Fuchsia spp.
Geranium sp.
Gladiolus sp.
Hedera helix
Heliotropium laevis
Heliotropium arborescens
(*H. peruvianum*)
Hordeum sp.
Hydrangea sp.
Hydrocotyle prolifera
Ipomoea maxima
Iris spp.
Lilium elegans
Lilium speciosum
Lycium sp.
Lycopersicon sp.

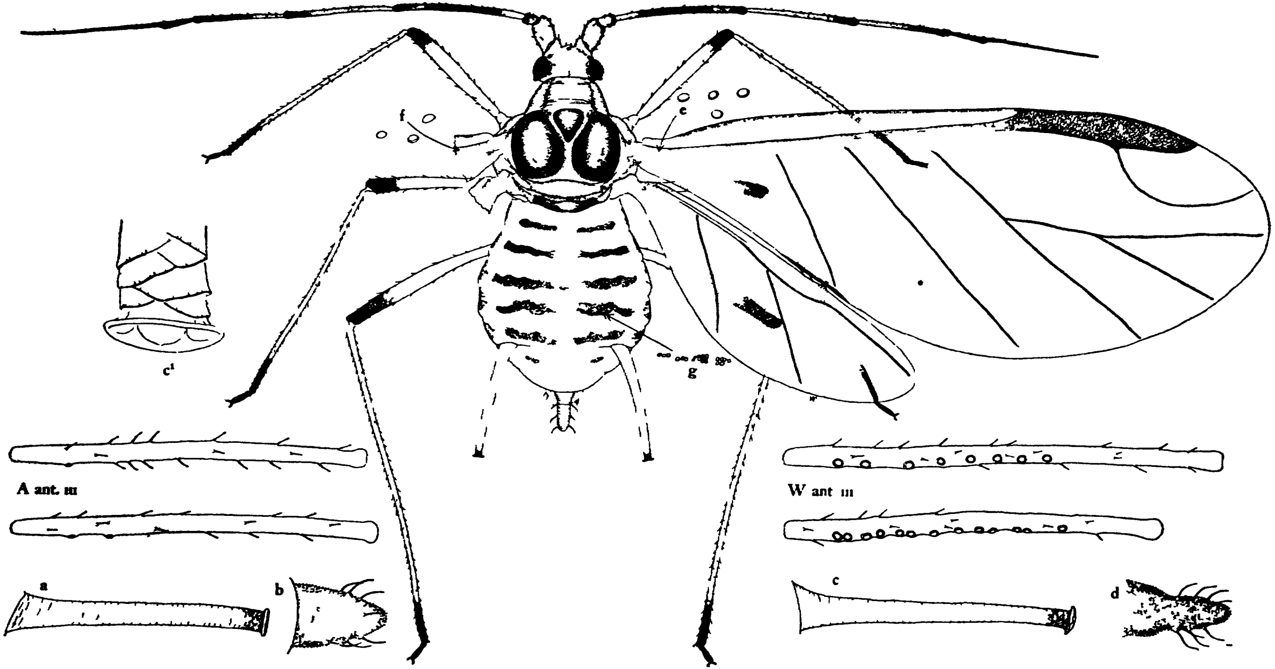

Fig. 4.—The foxglove aphid, *Myzus solani* (Kaltenbach), adult winged female; *W ant.*, antennal segment III; *c*, cornicle; *c*¹, tip of cornicle; *d*, cauda; *e*, and *f*, fenestras; *g*, gland pores as arranged in the dark transverse bands on the dorsum of the abdomen, *A ant.*, antennal segment III of apterous female, *a*, cornicle of apterous female; *b*, cauda of apterous female. (All greatly enlarged.)

Malvastrum coccineum
Myosotis sp.
Myrtus sp.
Nasturtium officinale
Nicotiana sp.
Orchis sp.
Oxalis sp.
Parthenocissa quinquefolia
Penstemon spectabilis
Persea sp.
Physalis peruviana
Plantago hirtella
Polymnia canadensis
Ranunculus acris
Ribes aureum var. *tenuiflorum*
Rorippa nasturtium-aquaticum
Rosa sp.
Rudbeckia laciniata
Rudbeckia laciniata
  var. *hortensia*
Rumex sp.
Sambucus sp.
Schizanthus sp.
Senecio cineraria
Senecio cruentus
  (*Doronicum cruentum*)
Senecio mikanioides
Senecio renifolius
  (*Cineraria renifolia*)
Senecio tenuifolius
Sinningia digitalifolia
  (*Gloxinia digitalifolia*)
Sisymbrium officinale
Solanum nigrum
Solanum tuberosum
Solidago missouriensis
Sparaxis sp.
Spiraea sp.
Stachys sp.
Steironema lanceolatum
Symphoricarpos albus
Streptocarpus sp.
  (*S. racemosus*)
Tradescantia fluminensis
Trifolium pratense
Triticum vulgare
Tropaeolum sp.
Tulipa spp.
Umbellularia californica
Vicia faba var. *equina*
Vinca major
Vinca minor
Viola nuttallii
Viola tricolor
Watsonia rosea
Zantedeschia aethiopia
Zantedeschia melanoleuca

THE FOXGLOVE APHID

Myzus solani (Kaltenbach, 1843, p. 15–16)
Aulacorthum solani (Kaltenbach)
Myzus convolvuli (Kaltenbach)
Macrosiphum aucubae Bartholomew (1932, p. 723–25)
Myzus gei Theobald (1919, p. 157; 1926, p. 349–50)
Myzus glaucii Theobald (1923, p. 103; 1926, p. 330–31)
Myzus hydrocotylei Theobald (1925, p. 73; 1926, p. 334–35)
Macrosiphum matsumuraeanum Hori (1926, p. 52–83)
Myzus mercurialis Theobald (1919, p. 158; 1926, p. 350–51)
Myzus polyanthi Theobald (1926, p. 341)
Myzus piceaellus Theobald (1916, p. 146; 1926, p. 315–16)
Myzus primulana Matsumura (1917, p. 400–1)
Myzus pseudolamii Theobald (1915*b*, p. 274; 1926, p. 345–46)
Myzus pseudosolani Theobald (1926, p. 313–15)
Macrosiphum veronicae Theobald (1913, p. 93)
Myzus veronicellus Theobald (1926, p. 347–48)
Aphis vincae Walker (1848, p. 429)

The foxglove aphid was so designated by Patch (1928) because of its importance as a pest of the common foxglove, *Digitalis purpurea,* a European ornamental extensively cultivated throughout much of North America. Along the Pacific Coast, especially in northern California and Oregon, this plant has become naturalized and is regarded as a pest of some importance on grazing lands. In these areas this host plant affords an abundance of food for the aphid.

This aphid may be distinguished from its nearest relatives by its more than medium size; its long antennae, which are much longer than the body; the large wings extending well beyond the tip of the abdomen; the dark, broken, transverse bands on the dorsum of the abdomen of the alates; and the long, almost cylindrical cornicles, which have a few broken concentric rings or reticulations around the apices. The apices are well flared and dusky black.

The apterous forms are usually without markings, except that in mounted specimens two longitudinal rows of groups of small dorsal glands are often evident on the sides of the abdomen. The prevailing colors may vary from whitish and yellowish, to pale or dark green with dusky areas at tips of the paler antennal segments, the apices of the leg segments, and the tips of the cornicles. (See figs. 4 and 5.)

The species is variable in size and color, a characteristic which has caused considerable confusion among aphidologists and resulted in the large number of synonyms. A fuller discussion of this aphid may be found in a previous paper (Essig, 1938*a*) and in one by Mason (1940).

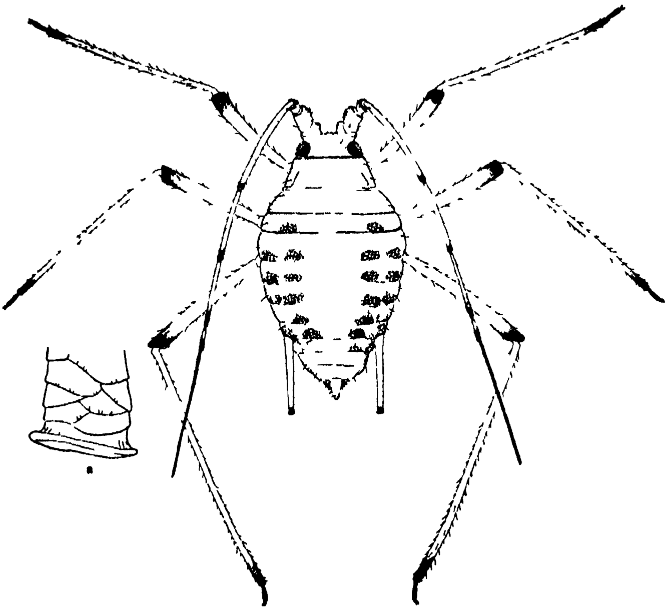

Fig. 5.—The foxglove aphid, *Myzus solani* (Kaltenbach), adult apterous female: *a*, tip of cornicle. Other details are shown in figure 5.

Nomenclature. In recent years this aphid has been most often designated as *Myzus convolvuli* (Kalt.) and *Myzus solani* (Kalt.). The Imperial Institute of Entomology, London, has adopted *Macrosiphum solani.* This specific name has page precedence over *convolvuli* in Kaltenbach's work (1843, p. 15–16, 40–41). In this country the genus *Myzus* is preferred to *Macrosiphum* for this species.

The genus *Aulacorthum* was erected by Mordvilko in 1914 (1914, p. 68) and has been accepted for this species by Hille Ris Lambers and some other European aphidologists.

Myzus convolvuli (Kalt.) is the name that has been most frequently used for this aphid in North America. It has now been accepted as a synonym of *M. solani* (Kalt.) in Europe and by many in this country.

Distribution. The foxglove aphid is widely distributed in the temperate regions and occurs in greenhouses in even colder areas. It is reported from the following places:

Africa: Egypt.

Asia: China, Japan.

Europe: Belgium, England, France, and Italy.

North America: Canada, in British Columbia (Victoria) and Ontario (Vineland); United States, in California (Berkeley, Lagunitas, Lompoc, Los Angeles, Niles, Pepperwood, Point Reyes, Riverside, San Francisco, San Jose, Shafter, Stanford University, Ukiah, Ventura, Vichi Springs, Woodacre).

South Pacific: Territory of Hawaii (Hawaii, Oahu, and Maui).

Host Plants. The foxglove aphid is an omnivorous feeder and has been reported as feeding upon many hosts. Doubtless in some instances the insects were merely casuals which may have been only transitory visitors. The list compiled to date includes the following:

Acer negundo
Althaea sp.
Amaranthus spp.
Ambrosia sp.
Anemone sp.
Anthemis sp.
Anthirrhinum spp.
Apium graveolens
Aquilegia formosa
Arctium lappa
Asarum sp.
Asclepias spp.
Atropa belladonna
Aucuba japonica
Auricula sp. (*Primula auricula?*)
Begonia spp.
Brassica spp.
Capsella bursa-pastoris
Carum spp.
Castilleja latifolia
Cestrum spp.
Chenopodium album
Chrysanthemum spp.
Cineraria spp. (*Senecio* spp.?)
Citrus maxima
Clintonia andrewsiana
Conium spp.
Convolvulus sepium
Convolvulus tricolor (*C. minor*)
Coprosma baueri
Crataegus spp.
Crotalaria anagyroides
Cucurbita pepo
Digitalis purpurea
Epipactis gigantea
Eriodictyon californicum
Erodium cicutarium
Erodium moschatum
Euphorbia boolceri
Fragaria spp.
Fuchsia arborescens
Galium verum (*G. luteum*)
Geranium spp. (including *G. dissectum*)
Geum aleppicum var. *structum*
Geum urbanum
Gladiolus spp.
Glaucium flavium (*G. luteum*)
Glycine max (*G. soja*)
Gnaphalium spp.
Helianthus tuberosus
Helichrysum sp.
Heracleum lanatum
Hieracium aurantiacum
Hieracium mucorum
Hydrangea spp.
Hydrocotyle vulgaris
Hyoscyamus sp.
Hypochaeris sp.
Ipomoea purpurea (*Convolvulus major*)
Lactuca sativa
Lamium purpureum
Lathyrus spp.
Leonotis leonurus
Lespedeza bicolor
Lilium spp.
Lycopersicon esculentum
Lysimachia sp.
Malus communis (*M. sylvestris*)
Melilotus spp.
Mentha arvensis var. *piperascens*
Mentzelia lindleyi (*Bartonia aurea*)
Mimulus aurantiacus (*Diplacus aurantiacus, D. glutinosus*)
Musa paradisiaca
Nemophila heterophylla
Oenothera spp.
Oxalis spp.
Phalaenopsis sp.
Phaseolus angularis
Phaseolus vulgaris
Physalis sp.
Picea abies (*P. excelsa*) (casual?)
Pisum sativum
Pittosporum spp.
Plantago sp.
Portulaca sp.
Potentilla sp.
Primula polyantha (*Polyanthus* sp.)
Primula sieboldii
Primula vulgaris
Radicula curvisiliqua
Ranunculus spp.
Rubus hawaiensis
Rubus idaeus
Rubus idaeus var. *strigosus*
Rubus laciniatus
Rumex acetosa
Rumex crispus
Sambucus racemosa
Sanicula menziesii
Senecio spp.
Solanum giganteum

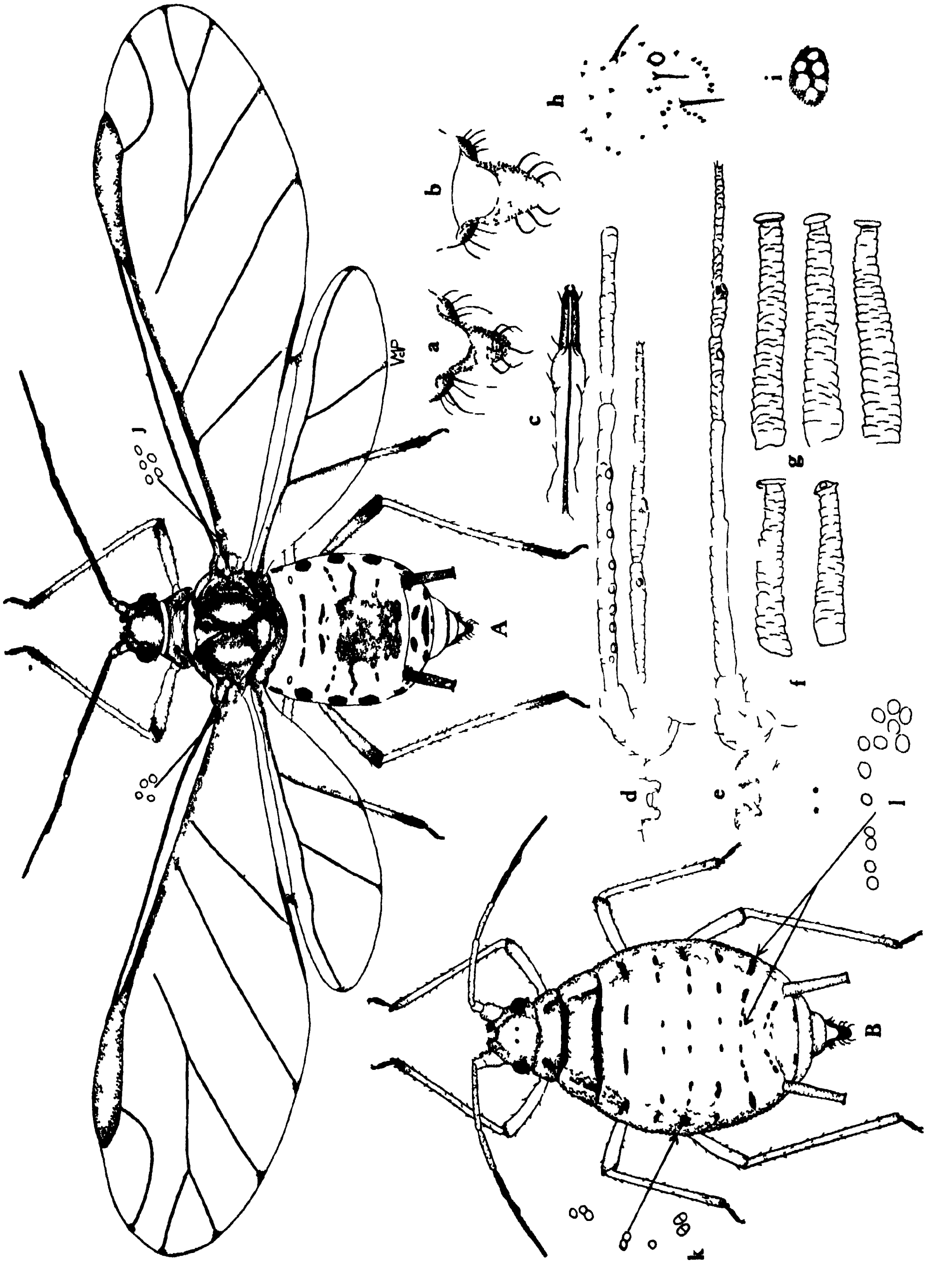

Fig. 6.—The ornate aphid, *Myzus ornatus* Laing: *A*, adult alate female, *a*, cauda; *c*, rostrum; *d*, front of head and antenna; *f*, cornicles; *h*, lateral abdominal dusky area, *i*, group of abdominal gland pores; *j*, fenestras near base of subcostal vein, *B*, adult apterous female; *b*, cauda; *e*, front of head and antenna; *g*, cornicles; *l*, gland pores arranged in dark broken bands on abdomen; *k*, lateral gland pores. (All greatly enlarged) (*From* Pan Pacific Entomologist 14.94, 1938)

Solanum jasminoides
Solanum melongena
Solanum tuberosum
Solidago sp.
Sonchus spp.
Stachys ajugoides
Stachys bullata
Stachys californica
Stellaria media
Symphoricarpos albus
Tanacetum sp.
Taraxacum officinale
Trifolium incarnatum
Trifolium pratense
Trifolium repens
Ulmus sp.
Urtica dioica
Urtica gracilis
Verbascum sp.
Verbena sp.
Veronica beccabunga
Vinca major
Vinca minor
Viola spp.
Wisteria sinensis
Zantedeschia sp.
Zanthoxylum pterota
(*Z. fagara*).

THE ORNATE APHID

Myzus ornatus Laing (1932, p. 52–53; Essig, 1938*a*, p. 92–95; Mason, 1940, p. 14–15)

The ornate aphid, *Myzus ornatus* Laing is very small and is quite easily recognized by the transverse, dusky, broken dark bands, on the dorsum of the apterae, as shown in figure 6. A more complete discussion of his aphid is given in a previous paper (Essig, 1938*b*).

Distribution. The ornate aphid is a newcomer in California, having first been taken in Berkeley, April.4, 1936, by the writer. It is rather widely distributed in Northern Europe. It was first reported in England in 1932 (Laing, 1932, p. 52–53). Since then it has been collected in Scotland, Ireland, and Belgium (Essig, 1938*a*). In 1941 it was discovered in New South Wales by Zeck (1941). In Honolulu, Hawaii, it was first collected in 1944 by E. C. Zimmerman, who also found it on the Island of Maui, 1945. In the United States it is now known to occur only in California and Oregon. In California it has been collected at Alton (H. T. Osborn), Berkeley (in greenhouses and outdoors, E. O. Essig), Lompoc (R. M. Bohart, K. F. Baker, W. C. Snyder), Los Angeles (R. H. Smith), and Sacramento (J. B. Steinweden); in Oregon, at Portland and Yachats (J. H. Schuh).

Host Plants. A list of known host plants follows:

Achyranthes spp.
Apium graveolens
  var. *dulce*
Artemisia vulgaris
Baccharis viminea
Bellis perennis
Brassica spp.
Buddleia orientalis
Calceolaria sp.
Camellia sp.
Capsicum frutescens
Chrysanthemum sp.
Cineraria sp.
  (*Senecio* sp.?)
Clematis sp.
Coleus sp.
Crepis virens
Crotalaria anagyroides
Dianthus barbatus
Digitalis purpurea
Dipsacus fullonum
Erodium sp.
Fragaria sp.
Fuchsia elegans
  (*F. globosa*?
  F. coccinea?)
Geum urbanum
Hedera helix
Helenium peregrinum
Helianthemum sp.
Heliotropium arborescens
Hydrangea sp.
Ilex sp.
Lantana sp.
Lapsana communis
Loiseleuria procumbens
  (*Azalea procumbens*)
Malus communis
  (*Pyrus malus*)
Matricaria chamomilla
Medicago sativa
Nasturtium officinale
  (*Roripa nasturtium*)
Panax lancasteri
Picris sp.
Plantago major
Potentilla sp.
Primula spp.
Ranunculus repens
Rheum rhaponticum
Richardia rehmanni
Rubus procerus
Rumex acetosella
Rumex obtusifolius
Salvia sp.
Scabiosa sp.
Taraxacum officinale
Teesdalia nudicaulis
Trifolium pratense
Ulmus procera
Urtica dioica
Valeriana sp.
Veronica spp.
Viola sp.

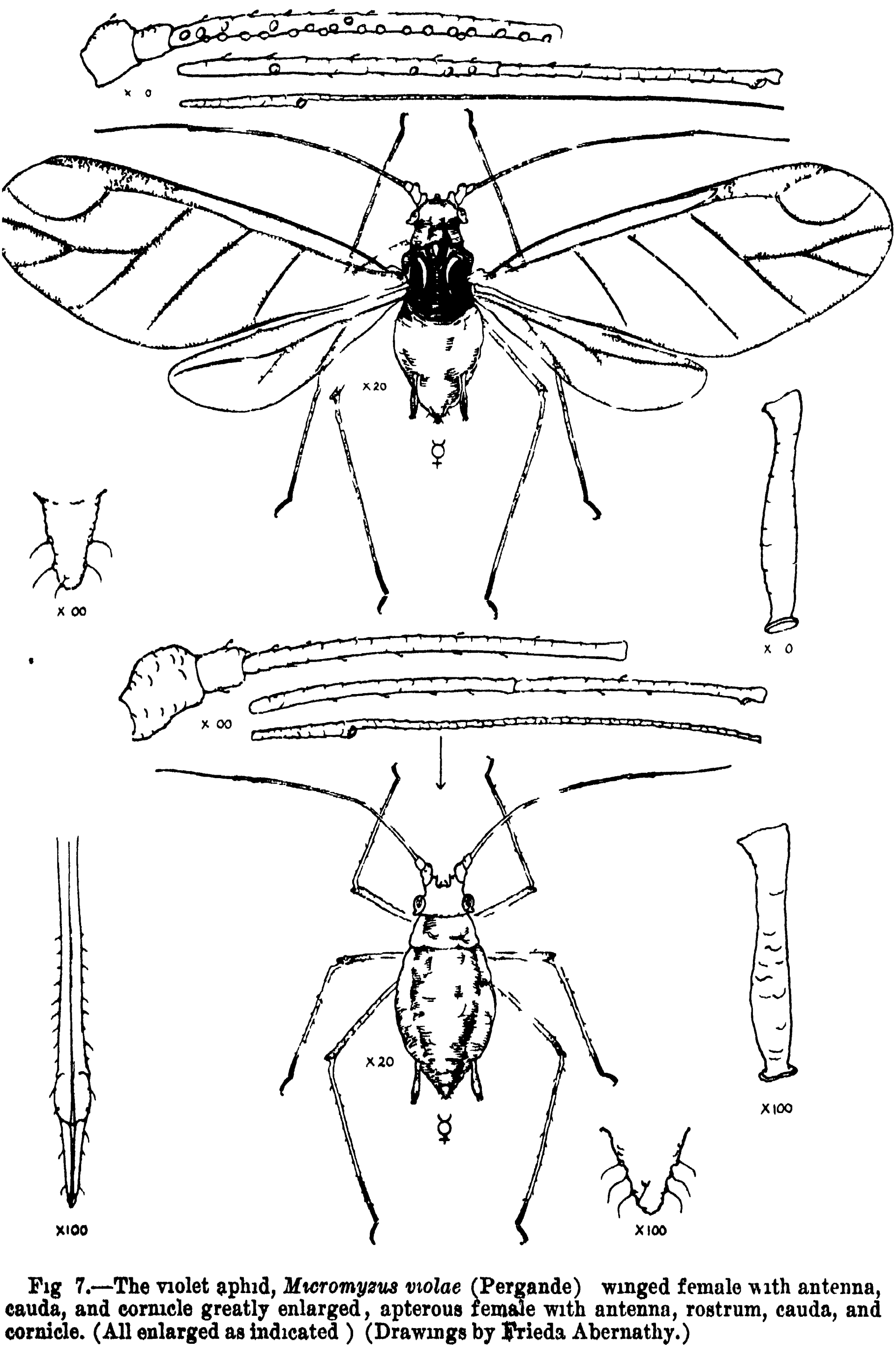

Fig 7.—The violet aphid, *Micromyzus violae* (Pergande) winged female with antenna, cauda, and cornicle greatly enlarged, apterous female with antenna, rostrum, cauda, and cornicle. (All enlarged as indicated) (Drawings by Frieda Abernathy.)

THE VIOLET APHID

Micromyzus violae (Pergande)
Rhopalosiphum violae Pergande (1900, p. 29–30)
Rhopalosiphum violae Essig (1909, p. 4–8)
Fullawayella violae (Pergande) Baker, 1919, p. 45–46; Takahashi, 1921, p. 29; Hardy, 1931, p. 34–35)
Neotoxoptera violae Theobald (1915*a*, p. 131–32; Baker, 1919, p. 45–46)

The violet aphid may at once be recognized by the pictured or cloudy wings and its very dark red, maroon, brownish, or even greenish-black body. The long antennae, slightly swollen cornicles, and short cauda also aid in separating it from other species infesting violets and pansies. It is slow and sluggish and appears to move only with considerable exertion. For more extended descriptions see Sanborn (1904, p. 64–65) and Essig (1935, p. 157). The winged and apterous females are shown in figure 7.

Nomenclature. Although this species has been shifted among at least four genera, the genus *Micromyzus* van der Goot (1916) now appears to be most logical.

The species *Rhopalosiphum violae* Essig was described without knowledge of Pergande's description. It is a synonym.

The genus *Neotoxoptera* was erected for what appears to be a variant of *Micromyzus violae* (Pergande). Specimens of this species frequently diverge from the type.

Distribution. The violet aphid has become widely distributed through commerce and now occurs in many parts of the subtropical and temperate regions where violets and pansies are cultivated or grow wild in the open. The aphid also abounds on the host plants grown in greenhouses or in other sheltered places. It has been reported from the following localities:

Africa: Onderstepoort, Transvaal.
Asia: Formosa.
Australia: Queensland.
North America: British Columbia; California (Claremont, Berkeley, Porterville, Riverside, Santa Ana, Santa Paula, Stanford University, Ventura), Connecticut, Illinois, Kansas, Mississippi, New Jersey, New Mexico, New York, and Washington.

Host Plants. The violet aphid feeds chiefly on the tender new growth of violets and pansies, including the leaves, leaf stems, and flowers; when abundant, it may occur on the entire plants and especially on the undersides of the leaves close to the ground. Infested plants often appear dwarfed, the foliage curled and sickly—no doubt the result of the virus diseases transmitted by the aphid. The only recorded host plants of this aphid are:

Lilium spp.
Mazus japonicus
Viburnum sp.
Viola spp.—many, but apparently not all species, are infested.

Adult alates may be found resting on many other plants upon which young are not reared.

OTHER SPECIES OF APHIDS REPORTED AS FEEDING ON VIOLETS AND PANSIES

Species of aphids feeding on violets and pansies and on other members of the family Violaceae are not numerous. In addition to the species already discussed, the following aphids have also been reported by Patch (1928, p. 180–81) as feeding upon these plants:

The buckthorn aphid, *Aphis abbreviata* Patch, on *Viola tricolor;* Maine (Patch, 1924, p. 38). Patch has made this a synonym of *Aphis rhamni* Boyer de Fonscolombe (1841, p. 177).

Aphis certa Walker on *Viola tricolor* England (Theobald, 1929, p. 63).

Aphis malvae (Koch) on *Viola tricolor;* Lahore (Das, 1918, p. 273).

Aphis malvacearum v.d. Goot (= *A. malvoides* Das) on *Viola tricolor;* Lahore (Das, 1918, p. 273).

Aphis plantaginis Schrank on *Viola* sp.; England, Belgium, France, Germany, Italy (Theobald, 1927, p. 131).

Aphis violae Schouteden on *Viola tricolor;* Belgium (Schouteden, 1900, p. 127; 1906, p. 229).

Macrosiphum malvae (Mosely) on *Viola odorata* and *V. tricolor;* England and Italy (Macchiati, 1883, p. 230; Theobald, 1926, p. 124). Theobald considers this species to be a synonym of *Macrosiphum pelargonii* (Kalt.). This latter species has so often been confused with *Myzus solani* (Kalt.) that it is possible *Macrosiphum malvae* may be a synonym of it.

The tulip leaf aphid, *Rhopalosiphoninus tulipella* (Theobald), on *Viola* sp.; England (Theobald, 1926, p. 222). This species has been found on tulip bulbs from Washington state (Essig collection, 1934), and alates resting on *Hesperis matronalis* at Corvalis, Oregon, April 15, 1941, by J. Schuh (Essig collection). It has been taken in quarantine on tulip bulbs from Holland (California State Department of Agriculture, March 6, 1945).

The green peach aphid, *Myzus persicae* (Sulzer), has been successfully used by H. H. P. Severin in transmitting celery calico to pansies. This insect does not appear to have been recorded as regularly feeding and reproducing on pansies and violas, although it has been listed on more than 200 host plants. It has been fully discussed by the author in a previous paper (Essig 1938*a*, p. 482–85).

Saltusaphis inscssa Walker reported on *Viola* sp. by Walker in England, represents perhaps casual visitors on violets or an error (Theobald 1927, p. 199). This aphid was subsequently taken on *Juncus maritima* in England.

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H I L G A R D I A

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EFFECTS OF POTASH DEFICIENCY AND EXCESS ON ORANGE TREES[1,2]

H. D. CHAPMAN,[3] S. M. BROWN,[4] AND D. S. RAYNER[5]

INTRODUCTION

MANY FIELD TRIALS to determine whether citrus would respond to potash have been made in California. No clear-cut indications of the need of potash have come from the tests (Parker and Batchelor, 1942).[6] Despite these results, many experienced growers are convinced that potash is indispensable to the maintenance of good quality or production or both, and persist in its use. In an effort to throw further light on the problem, a systematic study was begun by the authors some years ago. Work consisted first in determining the amounts of total, exchangeable, and water-soluble potassium in commercial orchards as compared with the amounts in adjacent nonfertilized areas.[7] This survey revealed wide differences in the potash content of citrus orchard soils, suggesting that in some groves potash might be deficient and that in others it might have accumulated to the point of excess.

Subsequent efforts were therefore directed toward three objectives: (1) to determine the characteristic effects of potash deficiency and excess on bearing citrus trees; (2) to determine the extent to which fruit quality is influenced by potash variations; and (3) to determine whether the potash content of the leaf or of any other part of the citrus tree is a reliable index of potash deficiency or excess.

An exploratory greenhouse experiment with citrus seedlings and cuttings to determine the effects of potash deficiency under a variety of nutrient conditions was carried out (Chapman and Brown, 1942*b*). This was followed by an outdoor experiment with bearing trees, and it is with this latter experiment that the present paper is concerned.

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[1] Received for publication October 29, 1946.

[2] Paper No. 563, University of California Citrus Experiment Station, Riverside, California.

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[6] See "Literature Cited" for citations, referred to in the text by author and date.

[7] Unpublished data on file in the Division of Soils and Plant Nutrition, University of California Citrus Experiment Station.

REVIEW OF LITERATURE

To the authors' knowledge no one has grown bearing citrus trees in controlled nutrient media of variable potassium supply over a long enough period to determine the effects of both potash deficiency and excess on the growth, foliage, composition, and fruit quality. Reed and Haas (1923) grew young orange trees in sand culture without potassium (sodium was substituted for potassium) for 17 months. Only the very earliest stages of potassium deficiency developed during this period. In describing these early symptoms, the authors state that some of the leaves produced were undersized and that the chlorophyll in some was faded. These effects were more pronounced on the south side of the tree. Some iron chlorosis was also seen. Haas (1936) later grew young citrus trees in sand cultures lacking potassium. He noted some scorching of leaves, twig dieback, and gum exudation on the wood. Some of the leaves showed resinous spots. No fruit was produced on these trees. Bryan (1935) grew grapefruit seedlings in potassium-deficient cultures and noted first a stimulation of growth, followed later by retardation. There was leaf puckering, and the fading of chlorophyll in small irregular spots on the leaf. Bryan also noted pustularlike brown spots on the leaves. The branches drooped and showed a lack of rigidity. Eckstein, Bruno, and Turrentine (1937), in their compilation of data on potash deficiency, cite leaf puckering along the midrib, and brown necrotic spots on leaves as indications of potassium lack in citrus. They also indicate that fruit from trees minus this element has thick, rough rind, and that its keeping quality is impaired.

In a Florida field experiment with oranges on Norfolk sand, Bahrt and Roy (1940) report that lack of potassium reduced yields and diminished fruit sizes. The potassium-deficient fruit had thinner rinds, contained less acid, but was slightly higher in soluble solids than fruit from trees receiving potash. Roy (1945), studying the juice characteristics of fruit from these plots, found less vitamin C in the potassium-deficient fruit although the difference was very slight (0.48 compared with 0.54–0.55 mgm per ml). He also found less citric acid, more reducing and total sugars, and less potassium in the juice of potassium-deficient oranges.

Young (1917), in California, found that nitrogen slightly decreased sugars, and very slightly increased acidity, but that phosphorus and potassium were without effect. Webber, writing in 1895 on early-day beliefs of growers in Florida, stated that potassium increased the acidity of fruit and lowered the sugar content. In the analysis of Florida fruit sent in from groves receiving low- and high-potassium fertilization, Collison (1913) found acidity to be slightly increased in the fruit from potash-treated trees. Camp, Gaddum, and Stahl (1933), in studies of Florida grapefruit under cold storage, concluded that potassium increased acidity and decreased sugar. Van der Plank and Turner (1936) found in fertilizer experiments with oranges in the western Transvaal, South Africa, that while without effect on yield, potassium sulfate increased fruit acidity. Anderssen (1937), reporting on a citrus fertilizer experiment in South Africa, found that, while potash was without effect on yield, juice acidity was increased, as was rind thickness. Allwright (1938), reporting on fertilizer experiments in the western Transvaal, South Africa, states that potassium sulfate increased fruit acidity. Neetles and Forsee (1941), reporting on an orange fertilizer experiment on a peat soil in Florida, found that fruit sizes were increased by potash.

Morris (1937), reporting on field fertilizer experiments with oranges in southern Rhodesia, South Africa, noted that increased fruit acidity resulted from potash additions. There was no significant effect on soluble solids.

Provan (1938), in connection with field fertilizer trials with navel oranges in Australia, found no evidence of an effect of potash on fruit quality.

Benton and Stokes (1931) report that in field experiments with oranges in Australia, a lack of potash resulted in smaller-sized fruit. These fruits were smoother, on the average, than fruit from potash-treated trees. The juice from fruit of potash-treated trees was lower in sugars and higher in nonsugars. Parker and Batchelor (1942), reporting on a number of fertilizer experiments in California, state that no significant effects on yield or on the commercial quality of fruit were obtained from applications of potash.

Relative to potassium excess, the authors know of no data with bearing citrus trees, but foliage symptoms on seedlings and cuttings grown in nutrient media have been described by several investigators (Chapman, Liebig, and Vanselow, 1939; Haas, 1932; Oppenheimer and Mendel, 1938).

EXPERIMENTAL METHODS

Materials and Technique. For this experiment a group of four-year-old orange trees (Valencias and Washington Navels on sour rootstock) were grown in water culture in large concrete containers of 700-liter capacity. The trees were originally procured from a commercial nursery as one-year-old budded trees, and were grown from June, 1936, to June, 1940, in 25-gallon sand cultures. By 1940 they had outgrown the sand cultures and were transferred to the concrete containers. These containers were cylindrical cement conduits provided with cement bottoms, and were painted inside ~~and~~ out with an asphaltum product. They were three quarters buried in the ground. Painted cement lids in two semicircular sections, with a semicircular hole at the center of each to accommodate the tree trunk, were used as covers.

Twenty trees in all were used to start with, but, during the six-year-period of this experiment (June, 1940, to June, 1946), five of the trees were discarded for one reason or another. At the outset, the trees were divided into three groups: one group received potassium sufficient to maintain concentrations between 3 and 7 p.p.m.; another, 30 to 40 p.p.m.; and the third, 300 to 400 p.p.m. (referred to in table 1 as 7, 39, and 390 p.p.m., respectively). During the first two years, these concentrations had to be adjusted somewhat, but from 1942 to 1946 all were held at constant potassium levels. In order to present for each tree the potassium-nutrition and tree-condition changes which obtained during the period of this experiment, a chronology for each of the trees is given in table 1. The 390 p.p.m. potassium concentration proved excessive, and the trees given this treatment were subsequently adjusted downward to 117 p.p.m. Trees originally in the 39 p.p.m. group were adjusted upward to 117 p.p.m. in April, 1942. Some of the trees of the 3 to 7 p.p.m. group were reduced to 1.5 p.p.m. and when, after a year, no sign of potassium deficiency developed, were further reduced to 1 p.p.m. As will be seen in table 1, begin-

TABLE 1

Chronology Showing Potassium Changes in Nutrient Medium and Tree Condition

Tree no. and variety	June 1, 1940 to Apr. 5, 1941		Apr. 5, 1941 to Apr. 5, 1942		Apr. 5, 1942 to Apr. 5, 1943		Apr. 5, 1943 to Apr. 5, 1944		Apr. 5, 1944 to Apr. 5, 1945		Apr. 5, 1945 to June 1, 1946	
	K	Tree condition	K	Tree condition	K	Tree condition	K	Tree condition	K	Tree condition	K	Tree condition
	p.p.m.		*p.p.m.*		*p.p.m.*		*p.p.m.*		*p.p.m.*		*p.p.m.*	
6, Valencia	7.0	Healthy	0.0	Slight K deficiency	0.0	Severe K deficiency	0.0	Severe K deficiency	0.0*	Moderate K deficiency	0.0	Moderate K deficiency
25, Valencia	39.0	Healthy	7.0	Healthy	0.0	Slight K deficiency	0 0	Severe K deficiency	0.0	Severe K deficiency	0.0	Severe K deficiency
23, Valencia	39.0	Healthy	7.0	Healthy	0.0	Slight K deficiency	0.0	Severe K deficiency	0.0	Severe K deficiency	0.0	Severe K deficiency
15, Washington Navel	7.0	Healthy	7.0	Healthy	0.0	Healthy	0.0	Severe K deficiency	0.0	Severe K deficiency	0.0	Severe K deficiency
13, Valencia	7.0	Healthy	1.5	Healthy	1.0	Healthy	1.0	Faint K deficiency	1.0	Faint K deficiency	1.0	Faint K deficiency
14, Valencia	7.0	Healthy	1.5	Healthy	1.0	Healthy	1.0	Faint K deficiency	1.0	Faint K deficiency	1.0	Faint K deficiency
4, Washington Navel	7.0	Healthy	1.5	Healthy	1.0	Healthy	1 0	Healthy	1.0	Faint K deficiency	1.0	Faint K deficiency
19, Washington Navel	7.0	Healthy	7.0	Healthy	1.0	Healthy	1.0	Healthy	1.0	Faint K deficiency	1.0	Faint K deficiency
22, Washington Navel	7.0	Healthy	1.5	Healthy	1 0	Healthy	1.0	Healthy	1.0	Healthy	1.0	Healthy
18, Valencia	7.0	Poor, root rot	7.0	Healthy	....	Discarded†	....	....	....	....	....	....
16, Washington Navel	39.0	Poor, discarded†	....	....	....	....	....	....	....	....	....	....
2, Valencia	39.0	Healthy	39.0	Fair	117 0	Healthy	117.0	Healthy	117 0	Healthy	117.0	Healthy
8, Valencia	39.0	Healthy	39.0	Healthy	117.0	Healthy	117.0	Healthy	117.0	Healthy	117.0	Healthy
7, Valencia	390.0	Moderately healthy	390.0	Poor‡	117.0	Moderately healthy	117.0	Moderately healthy	117.0	Healthy	117.0	Healthy
26, Valencia	39.0	Healthy	390.0	Moderately healthy	117.0	Moderately healthy	117.0	Healthy	117.0	Healthy	117.0	Healthy
3, Washington Navel	390.0	Healthy	390.0	Nearly dead	117.0	Discarded‡	....	....	....	....	....	....
24, Washington Navel	390.0	Healthy	390.0	Poor‡	117.0	Poor	117.0	Recovering	117.0	Healthy	117.0	Healthy
10, Washington Navel	39.0	Healthy	39.0	Fair	117.0	Poor	117.0	Healthy	117.0	Healthy	117.0	Healthy
1, Valencia	390.0	Poor, discarded†	....	....	....	....	....	....	....	....	....	....
21, Valencia	390.0	Healthy	390.0	Discarded†	....	....	....	....	....	....	....	....

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* Potassium was accidentally added to this culture in the spring of 1944 but was present only a few days. Enough was absorbed to bring about some recovery.

† In some trees, sand pockets were inadvertently left in the thickened matted ball; and on transfer to the solution cultures, the roots in these pockets rotted. When this was discovered, the necrotic roots were cut away. Some of the trees recovered and others had to be discarded.

‡ All of the trees receiving 390 p.p.m. potassium began to show more or less distress in about 6 months after the change to this concentration; several of them developed severe brown-rot gummosis and were finally discarded.

TABLE 2

COMPOSITION OF NUTRIENT SOLUTIONS

Tree no. and dates	Concentration in milliequivalents per liter*								Concentration in p.p.m.		
	Ca	Mg	K	Na	NO_3	PO_4	SO_4	Cl	B	Mn	Al
	m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Tree 6:											
June 1, 1940, to April 5, 1941	12.11	5.50	0.20	1.37	10.00	0.30	8.51	0.57	1.0	0.5	0.20
April 5, 1941, to April 5, 1942	12.11	5.50	0.00	0.60	9.97	0.30	7.64	0.50	1.0	0.5	0.20
April 5, 1942, to April 5, 1943	12.11	5.50	0.00	0.60	9.97	0.30	7.64	0.50	2.0	0.5	0.20
Tree 23:											
June 1, 1940, to April 5, 1941	8.00	4.00	1.05	1.37	1.00	0.30	12.65	0.57	1.0	0.5	1.10
April 5, 1941, to April 5, 1942	12.11	5.50	0.20	1.37	10.00	0.30	8.51	0.57	1.0	0.5	0.20
April 5, 1942, to April 5, 1943	12.11	5.50	0.00	0.00	9.97	0.30	7.64	0.00	2.0	0.5	0.30
Tree 25:											
June 1, 1940, to April 5, 1941	8.00	4.00	1.05	1.37	1.00	0.30	12.65	0.57	1.0	0.5	1.0
April 5, 1941, to April 5, 1942	12.11	5.50	0.20	1.37	10.00	0.30	8.51	0.57	1.0	0.5	1.0
April 5, 1942, to April 5, 1943	12.11	5.50	0.00	0.60	9.97	0.30	7.64	0.50	2.0	0.5	1.0
Tree 15:											
June 1, 1940, to April 5, 1941	12.11	5.50	0.20	1.37	10.00	0.30	8.51	0.57	1.0	0.5	1.0
April 5, 1941, to April 5, 1942	12.11	5.50	0.20	1.37	10.00	0.30	8.51	0.57	1.0	0.5	1.0
April 5, 1942, to April 5, 1943	12.11	5.50	0.00	0.00	9.97	0.30	7.64	0.00	2.0	0.5	1.0
Trees 6, 23, 25, and 15:											
April 5, 1943, to June 1, 1946	13.31	5.50	0.00	0.60	9.97	1.50	7.64	0.50	1.0	0.5	1.0
Tree 19:											
June 1, 1940, to April 5, 1942	12.11	5.50	0.20	1.37	10.00	0.30	8.51	0.57	1.0	0.5	1.0
April 5, 1942, to April 5, 1943	12.11	5.50	0.0025	0.00	9.97	0.30	7.64	0.00	2.0	0.5	1.0
Trees 4, 22, 13, and 14:											
June 1, 1940, to April 5, 1941	12.11	5.50	0.20	1.37	10.00	0.30	8.51	0.57	1.0	0.5	1.0
April 5, 1941, to April 5, 1942	12.11	5.50	0.037	0.60	9.97	0.30	7.64	0.50	1.0	0.5	1.0
April 5, 1942, to April 5, 1943	12.11	5.50	0.0025	0.00	9.97	0.30	7.64	0.00	2.0	0.5	1.0
Trees 4, 22, 13, 14, and 19:											
April 5, 1943, to June 1, 1946	13.31	5.50	0.0025	0.00	9.97	1.50	7.64	0.00	1.0	0.5	1.0
Trees 3, 24, and 7:											
June 1, 1940, to April 5, 1942	3.14	5.50	10.02	1.27	10.00	0.30	9.26	0.57	1.0	0.5	1.0
April 5, 1942, to April 5, 1943	9.41	5.50	3.00	1.37	10.00	0.30	8.51	0.57	2.0	0.5	1.0
Tree 26:											
June 1, 1940, to April 5, 1941	8.00	4.00	1.05	1.27	1.00	0.30	12.65	0.57	1.0	0.5	1.0
April 5, 1941, to April 5, 1942	3.14	5.50	10.02	1.27	10.00	0.30	9.26	0.57	1.0	0.5	1.0
April 5, 1942, to April 5, 1943	9.41	5.50	3.00	1.37	10.00	0.30	8.51	0.57	2.0	0.5	1.0
Trees 2, 8, and 10:											
June 1, 1940, to April 5, 1941	12.11	5.50	1.05	1.27	10.00	0.30	9.26	0.57	1.0	0.5	1.0
April 5, 1941, to April 5, 1942	12.11	5.50	1.00	1.37	10.00	0.30	9.31	0.57	1.0	0.5	1.0
April 5, 1942, to April 5, 1943	9.41	5.50	3.00	1.37	10.00	0.30	8.51	0.57	2.0	0.5	1.0
Trees 3, 24, 7, 26, 2, 8, and 10:											
April 5, 1943, to June 1, 1946	10.61	5.50	3.00	1.27	10.00	1.50	8.51	0.57	1.0	0.5	1.0

* The sum of the cations and anions is not exactly equal because acid phosphate was used as a source of phosphorus.

ning in April, 1942, four trees had been deprived of potassium completely; five trees were reduced to 1.0 p.p.m.; and seven trees were receiving 117 p.p.m. potassium. The potassium concentrations of the nutrient solutions as stated, were not changed from this time on.

The composition of the complete nutrient solutions supplied each tree from June, 1940, to June, 1946, are shown in table 2. In addition to the ions shown in table 2, iron was added three times a week at a rate each time to provide 0.1 p.p.m. Fe. The solutions were continuously aerated.

Water transpired by the tree was compensated for by additions of distilled water. The solutions were completely renewed at monthly intervals. This technique made it possible to maintain reasonably constant the concentration of all of the major ions. At no time did the trees show signs of zinc, copper, boron, or manganese deficiencies, but in winter some of the trees occasionally displayed slight iron chlorosis. This condition largely cleared itself as the solutions warmed up in the spring.

With nutrient solutions renewed once a month, the cultures were not maintained constantly at the levels designated. Analysis indicated that all of the potassium of the 1 p.p.m. cultures was absorbed in from one to two days. The technique followed with these cultures, therefore, was to add enough potassium to give 1 p.p.m. every week. With this technique, all but one of the trees in this group developed faint, but unmistakable, potassium-starvation symptoms. The 117 p.p.m. cultures actually varied between 90 and 117 p.p.m. potassium.

For the first three years of the experiment, the pH of the solutions was maintained at approximately pH 5.0, using NaOH or H_2SO_4 to adjust as needed. However, the cultures became infected with *Phytophthora parasitica* fungus, and the roots of some of the trees turned dark brown and made little new growth. In an effort to provide conditions less favorable for this organism, the pH of all the cultures was lowered in April, 1943, to pH 4.0. This change brought about a remarkable development of healthy roots, and from then on until the end of the experiment root appearance and growth were excellent.

In all of the cultures, determinations were run as often as required for phosphate, potassium, and nitrate; and appropriate adjustments were made.

Records of Tree Performance. Systematic notes of both root and top behavior were made at periodic intervals throughout the course of the experiment. Photographs, both black-and-white and colored, were taken from time to time of all the trees and of such abnormalities as occurred.

The fruit picked from each tree annually was weighed, counted, and graded for rind texture, stem, and stylar-end characteristics. A measure of average size was obtained by cutting each fruit transversely at its point of maximum diameter and measuring diameter in millimeters. At the same time, measurements were made of rind thickness. In most years a representative sample of fruit was ground in a Waring Blendor and analyzed for inorganic constituents. The remainder of the fruit was juiced, the juice was strained through a 2-mm sieve, and from the weight of the whole fruit and the juice, the percentage of juice was determined. Samples of juice were tested for total solids with a Brix hydrometer, and determinations of acid and vitamin C were made. The juice was also tasted for palatability and flavor. From the measurements [illegible] diameter, an average diameter for all of the fruit from a given tree was obtained; and this figure was converted into number of fruits per packed box as an index of size. No statistical studies were made; statements on significance are the authors' interpretation of the data.

RESULTS

EFFECT OF POTASSIUM DEFICIENCY

The experiment started with a group of vigorous and healthy four-year-old trees. One group was taken through successive stages from a low, but adequate, potash supply to extreme deficiency. Another group was taken from the same base to just the earliest stages of potash deficiency. A third group was taken from the same base to potash excess. Hence it has been possible to note in detail all of the growth, foliage, and fruit characteristics associated therewith. Since the potash nutrition of each group was constant for the last four years of the experiment it was possible to check on any seasonal or yearly changes in symptomatology. In going from potassium adequacy to deficiency, no material change in osmotic pressure of the solution was required; for the trees were maintained from the outset at a relatively low potash level.

Inorganic Composition of Leaves and Fruit. In the spring of 1941, and each year thereafter, a representative set of the newly emerged leaves from each tree was picked for analysis. Leaves of this same growth cycle were collected each succeeding month until April of the following year. Then in May of the new year the same procedure was repeated. With this record for each tree in the experiment, it has been possible to follow the progressive change in inorganic composition with the adjustment in potassium level and also the change, in any one year, of leaf composition with age. It is not necessary to present the entire leaf-analysis record here, but table 3 shows the year-to-year changes in leaf composition, and table 4 the monthly changes during 1944–45, for six trees. The trees had come into complete adjustment with the solution by 1944—hence, the monthly changes reflect the effect of age of leaf on composition. Reference to table 3 shows that by August, 1943, trees 25 and 15, lacking potash, had become relatively constant in inorganic composition. The potassium content of 4-month-old spring-cycle leaves from acutely potash-deficient trees fluctuated around 0.2 per cent (see also table 4). Trees 4 and 13, receiving 1 p.p.m. potassium, however, did not attain equilibrium until 1944. The potash content of leaves from these trees leveled off at around 0.3 per cent. Trees 2 and 24, receiving 117 p.p.m. potash, also failed to reach constant levels until 1944. The potash in leaves from these trees showed values of over 1.0 per cent. On the whole, a fairly good correlation between potassium content of the nutrient medium and leaf content has been found. The general relation between nutrient-solution level of potassium and that found in spring-cycle leaves from 4 to 7 months of age is as follows:

Potassium in culture solution, p.p.m.	Condition of tree	Range of potassium in leaves, per cent of dry weight
None	Acute potassium deficiency	0.14–0.24
0–1	Very slight deficiency; trees healthy in appearance	0.26–0.35
30–40	No deficiency; trees healthy	0.52–0.86
90–117	No deficiency; trees healthy	1.04–1.73
350–400	Trees stunted	2.15–3.63

The difference in percentage potash between leaves from trees acutely deficient in potash and trees showing barely perceptible symptoms is very narrow. But a difference of about 0.1 per cent of potassium in the leaf in this range makes a tremendous difference in tree appearance (compare plates 1 and 3).

TABLE 3

INORGANIC COMPOSITION OF SPRING-CYCLE LEAVES IN RELATION TO POTASSIUM CONTENT OF NUTRIENT MEDIUM

Date of sampling leaves	Tree 25, Valencia						Tree 15, Washington Navel					
	K conc.	Composition, per cent of dry matter					K conc.	Composition, per cent of dry matter				
		Ca	Mg	K	N	P		Ca	Mg	K	N	P
	p.p.m.	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*	*p.p.m.*	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*
Sept. 10, 1941	7.0	4.93	....	0.86		..	7.0	4.80		0.94	....	
Aug. 27, 1942	0.0	6.17	..	0.36	.	.	0.0	4.25	...	0.41	..	
Aug. 13, 1943	0.0	5.05	0.46	0.20	3.11	0.16	0.0	4.50	0.36	0.25	3.36	0.14
Aug. 15, 1944	0.0	5.41	0.48	0.24	4.09	0.18	0.0	4.75	0.46	0.21	4.06	0.16
Aug. 29, 1945	0.0	5.42	...	0.19	3.26	0.17	0.0	5.16	...	0.19	4.02	0.15

Date of sampling leaves	Tree 4, Washington Navel						Tree 13, Valencia					
	K conc.	Composition, per cent of dry matter					K conc.	Composition, per cent of dry matter				
		Ca	Mg	K	N	P		Ca	Mg	K	N	P
	p.p.m.	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*	*p.p.m.*	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*
Sept. 10, 1941	7.0	5.40	....	0.87	....	...	7.0	5.54	.	0.81	....	...
Aug. 27, 1942	1.5	4.73		0.43	.	.	1.5	4.60	..	0.40	....	....
Aug. 13, 1943	1.0	4.42	0.46	0.52	3.40	0.20	1.0	4.84	0.42	0.48	3.08	0.21
Aug. 15, 1944	1.0	5.26	0.46	0.34	2.79	0.13	1.0	6.66	0.44	0.31	2.78	0.14
Aug. 29, 1945	1.0	5.17	....	0.29	2.56	0.15	1.0	4.46	....	0.30	....	.

Date of sampling leaves	Tree 2, Valencia						Tree 24, Washington Navel					
	K conc.	Composition, per cent of dry matter					K conc.	Composition, per cent of dry matter				
		Ca	Mg	K	N	P		Ca	Mg	K	N	P
	p.p.m.	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*	*p.p.m.*	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*
Sept. 10, 1941	39.0	6.07	....	0.61	....	...	390.0	1.85	....	3.69	...	....
Aug. 27, 1942	39.0	4.66	....	0.99	...	....	390.0	2.15	....	2.40	....	....
Aug. 13, 1943	117.0	3.05	0.30	1.72	2.67	0.19	117.0	3.55	0.28	2.26	2.80	0.20
Aug. 15, 1944	117.0	4.36	0.28	1.17	2.94	0.15	117.0	3.40	0.34	1.43	3.08	0.15
Aug. 29, 1945	117.0	2.64	....	1.38	2.82	0.14	117.0	3.49	....	1.81	....	0.15

On the other hand, leaves accumulate large quantities of potash if it is present in excess in the nutrient medium—in other words, there is a point at which luxury consumption begins. The range of leaf potassium between extreme deficiency and extreme excess is between 20 and 30 fold.

With respect to the effects of varying potash on the amounts of other constituents, trees 25 to 15, severely affected by potassium deficiency, are much higher in calcium, magnesium, and nitrogen than are trees 2 and 24, which were given ample potassium. There is a slight indication of higher phosphorus,

TABLE 4

CHANGE IN COMPOSITION OF SPRING-CYCLE LEAVES WITH AGE IN RELATION TO POTASSIUM LEVEL OF THE NUTRIENT MEDIUM

Date of sampling leaves	Tree 25, Valencia						Tree 15, Washington Navel					
	K conc.	Composition, per cent of dry matter					K conc.	Composition, per cent of dry matter				
		Ca	Mg	K	N	P		Ca	Mg	K	N	P
	p.p.m.	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*	*p.p.m.*	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*
May 17, 1944	0 0	3.52	0.33	0.37	3 73	0.24	0.0	3.20	0.32	0.34	3.75	0.24
June 14, 1944	.0	4 06	.35	.21	3.65	.20	.0	3.60	.31	.23	4.28	.20
July 13, 1944	.0	5.29	.36	.24	4.12	.17	.0	4 76	.34	.25	4.22	.15
Aug. 15, 1944	.0	5.41	.48	.24	4 09	.18	.0	4.75	.46	.21	4 06	.16
Sept. 13, 1944	.0	5.80	.56	.22	3 84	.18	.0	4.71	.48	.22	3.76	.18
Oct. 16, 1944	.0	5.49	.56	.14	4 11	.20	.0	4 32	.49	.20	3.58	.19
Dec. 13, 1944	.0	6.00	57	.14	3.98	.18	.0	5 34	55	.16	3.22	.15
Feb. 9, 1945	.0	5.54	.56	.11	3.86	.18	.0	4 56	.50	.15	3 36	.16
Apr. 12, 1945	0	5 96	.60	.05	4 11	.18	0	5 28	56	11	4.26	.17
Average	*0.0*	*5.23*	*0.49*	*0.19*	*3.94*	*0.19*	*0.0*	*4 50*	*0.45*	*0.21*	*3.83*	*0.17*

Date of sampling leaves	Tree 4, Washington Navel						Tree 13, Valencia					
	K conc.	Composition, per cent of dry matter					K conc.	Composition, per cent of dry matter				
		Ca	Mg	K	N	P		Ca	Mg	K	N	P
	p.p.m.	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*	*p.p.m.*	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*
May 17, 1944	1 0	3 73	0.34	0 38	2.80	0.18	1.0	4.22	0.33	0.33	2.67	0.19
June 14, 1944	1 0	4 20	.28	.31	2.97	.18	1.0	5.16	.31	.31	2.78	.20
July 13, 1944	1 0	5.47	.34	.37	2.88	.14	1.0	6 37	.34	.33	2.73	.15
Aug. 15, 1944	1.0	5.26	.46	.34	2.79	.13	1.0	6.66	.44	.31	2.78	.14
Sept. 13, 1944	1 0	5.15	.53	.29	2.74	.16	1.0	6.30	.57	.26	2.66	.16
Oct. 16, 1944	1.0	5.79	.56	.30	2.89	.19	1.0	6.34	.57	.33	2.96	.19
Dec. 13, 1944	1.0	5.61	.57	.31	2.82	.16	1.0	6.42	.61	.35	2.79	.17
Feb. 9, 1945	1 0	5 36	.57	.28	3.01	.16	1.0	6 25	.56	34	2.86	.16
Apr. 12, 1945	1.0	5.38	.51	.27	2.75	.14	1.0	6.43	.57	.30	2.75	.14
Average	*1.0*	*5.10*	*0.46*	*0.32*	*2.85*	*0.16*	*1.0*	*6 02*	*0.48*	*0.32*	*2.77*	*0.17*

Date of sampling leaves	Tree 2, Valencia						Tree 24, Washington Navel					
	K conc.	Composition, per cent of dry matter					K conc.	Composition, per cent of dry matter				
		Ca	Mg	K	N	P		Ca	Mg	K	N	P
	p.p.m.	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*	*p.p.m.*	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*
May 17, 1944	117	3.25	0.32	1.35	2.56	0.17	117	2.96	0.34	1.75	2.61	0.17
June 14. 1944	117	3.50	.28	1.51	2.81	.19	117	3.45	.28	1.74	2.66	.18
July 13, 1944	117	4.34	.32	1.36	2.96	.15	117	3.66	.32	1.20	2.77	.16
Aug. 15, 1944	117	4.36	.28	1.17	2.94	.15	117	3.40	.34	1.43	3 08	.15
Sept. 13, 1944	117	4.02	.28	1.17	2.87	.14	117	3.68	.27	1.66	2.95	.16
Oct. 16, 1944	117	4.49	.26	1 29	2.88	.18	117	3.96	.31	1.73	2.98	.19
Dec. 13, 1944	117	4.46	.35	1.04	2.88	.15	117	3.72	.31	1.47	2.80	.15
Feb. 9, 1945	117	3.67	.32	1.01	2.91	.14	117	3.61	.28	1 55	2.88	.14
Apr. 12, 1945	117	3.80	.23	0.94	2.66	.09	117	3.71	.28	1.43	2.45	.11
Average	*117*	*3.99*	*0.29*	*1.20*	*2.83*	*0.15*	*117*	*2.57*	*0.30*	*1.55*	*2.80*	*0.16*

also, but this is of questionable significance. These results are consistent with analyses made of leaves from potash-deficient lemon and orange plants reported in an earlier paper (Chapman and Brown, 1942*b*).

Trees (nos. 4 and 13) only slightly deficient in potassium were significantly higher in calcium and magnesium than trees (nos. 2 and 24) receiving ample potassium; but the two groups showed no difference in total nitrogen.

The changes, with age, in inorganic composition of leaves are shown in the data of table 4. Very young leaves are characterized by a somewhat lower calcium and magnesium content than older leaves. During the period of July through October, there is a fair degree of constancy in composition, although, as might be expected, there are some fluctuations. In April, when the blossom and new cycle growth is emerging, there is in most cases a significant decrease in potassium content. On a percentage basis, calcium increases. The trend in magnesium, nitrogen, and phosphorus is not so clear, but in other research there has been noted, in general, a tendency for nitrogen and phosphorus to decrease along with potassium. This tendency suggests that at this stage of development, potassium, nitrogen, and phosphorus are translocated from older leaves to the newly developing parts.

TABLE 5

INORGANIC COMPOSITION OF FRESH ORANGES IN RELATION TO POTASSIUM SUPPLY

Potassium in nutrient solution, tree no., and variety	Condition of tree	Composition, per cent fresh weight				
		Ca	Mg	K	N	P
0 p.p.m. K:		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Tree 15, Washington Navel	Slight K deficiency	0.065	0.014	0.081	0.23	0.022
Tree 25, Valencia	Slight K deficiency	.061	.012	064	.21	021
Tree 23, Valencia	Slight K deficiency	.068	.012	051	.21	.030
1 p.p.m. K:						
Tree 4, Washington Navel	Healthy	065	.015	.095	22	020
Tree 22, Washington Navel	Healthy	.058	.015	.108	22	.025
Tree 19, Washington Navel	Healthy	.046	012	111	.24	023
Tree 13, Valencia	Healthy	.062	.015	.079	.20	.023
Tree 14, Valencia	Healthy	069	.012	.096	20	022
117 p.p.m. K:						
Tree 25, Washington Navel	Healthy	.041	012	.185	21	015
Tree 7, Valencia	Healthy	039	012	174	19	.022
Tree 2, Valencia	Healthy	.049	015	.163	.19	.022
Tree 8, Valencia	Healthy	0.048	0.014	0.166	0.21	0.016

The inorganic composition of mature oranges produced under conditions of variable potassium supply is shown by the data in table 5. Fruit from potassium-deficient trees showed less of this element and more calcium than fruit from trees receiving ample potassium. No significant trend can be seen in the magnesium, nitrogen, or phosphorus content, however.

Tree and Foliage Effects. In April, 1941, tree 6 (Valencia) was deprived of potassium completely. During the ensuing summer and fall, this tree made less vegetative growth. Some of the leaves on the south side became a little bronzed and sunburned; others showed a slight tendency to curl. Lack of new growth caused this tree, by fall, to have less foliage than the rest, although, in general, it had showed health and good color during the first year.

In April, 1942, at the time of blossoming, there was a greater fall of the old leaves from tree 6 than from the trees receiving potassium. Although a considerable amount of new growth appeared, there was in general less blossom. Many of the old leaves which dropped at this time were quite yellowish (see plate 4, *D*). Lack of growth in the preceding season and greater leaf fall caused this tree to become much more defoliated than the other trees.

The leaves of tree 6 during the summer and fall of 1942—15 to 18 months after potassium had been omitted from the culture solution—began to show pronounced curling and crinkling. They took on a thick leathery feel and many of them became rather yellowish. Such new growth as emerged from time to time was weak, the leaves were undersized, and some of the stems were at first somewhat yellow at the point of attachment with the larger twigs. This yellowish portion of the stem subsequently greened up but frequently showed an S-like curvature. The tendency for S-shaped laterals was not a pronounced symptom, nor did all emerging shoots show it.

The other trees subsequently deprived of potassium (trees 25, 23, 15) followed much the same course as tree 6, the symptoms of potassium lack coming on very slowly. By the spring of 1946, trees 25, 23, and 15 had reached a state of acute potassium deficiency. Tree 6, to which potassium had been accidentally added in the summer of 1944 (after which it made some recovery), was in a stage of semiacute deficiency; while trees 13, 14, 4, and 19 showed only very slight deficiency. Color pictures depicting these three stages are presented in plates 1, 2, and 3.

The acute stage (plate 1) is characterized, among other things, by pronounced dieback. The dieback of twigs and branches occurred, in the main, following the spring blossom and growth cycle. The foliage at this stage was yellowish, faded, and lusterless, but there was no definite or distinctive leaf pattern. As stated, severe crinkling, twisting, puckering, and cupping of leaves occurred. These leaves were leathery and thick. Bloom was sparse and new growth was limited. There was always an abnormal leaf fall after the spring flush of growth. With subacute deficiency, the yellowing and curled foliage was less pronounced, there was less dieback, and there was more foliage (plate 2).

Where the deficiency was slight (plate 3), there was very little, or nothing, definite or specific by way of foliage color and density, leaf size, or other characteristics by which a positive diagnosis could be made. Having for comparison trees sufficient in potash and, conversely, trees deficient, it was possible to note certain symptoms which indicated slight deficiency. There was a somewhat greater drop of leaves from these trees in the spring of the year than from those receiving sufficient potash. Many of the abscissing leaves were more yellowish than those of normal trees. Here and there could be seen a few crinkled leaves. However, it is always possible to find deformed or somewhat crinkled leaves even on healthy, normal trees; so that this symptom, by itself, is not a clear-cut indication of potassium deficiency. Another indication of potassium lack, which of itself would not be diagnostic for potassium, was a tendency toward less new growth. Another characteristic of these slightly deficient trees was the production of small-sized fruit, which colored earlier than fruit on the trees receiving ample potash. All of these symptoms, together with the low-potash content of the foliage (0.30 per cent as against 0.20 for the acutely deficient trees and over 1.0 per cent for the amply supplied trees) left no doubt that these trees, although on the whole entirely healthy in appearance, were slightly deficient in potassium.

To illustrate in greater detail the various foliage symptoms characterizing potassium deficiency, a group of color pictures are presented in plate 4. Plate 4, *B*, shows potassium-deficient Valencia fruit from tree 6 just turning color, in comparison with fruit (4, *A*) of the same age from tree 2 receiving ample potash. Note that the potassium-deficient fruit is small but smooth in rind texture. Some of the leaves on this shoot are puckered and somewhat faded but with no definite pattern. Plate 4, *C*, shows mature, potassium-deficient Valencia fruit together with further leaf characteristics. Plate 4, *D*, shows the yellow coloration and excessive shedding tendency of old leaves on a potassium-deficient tree after the spring bloom. Plate 4, *E*, *F*, shows the twisting, curling, and puckering of leaves on potassium-deficient trees, together with the yellowing and lack of definite leaf pattern.

TABLE 6

MINOR-ELEMENT CONTENT OF LEAVES AND FIBROUS ROOTS OF ORANGE TREES DEFICIENT AND AMPLY SUPPLIED WITH POTASH

(Expressed on a p.p.m. in dry-matter basis)

Element	Tree 25, K deficient		Tree 26, ample K	
	Roots	Leaves	Roots	Leaves
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
Silver	0.4	0.7	0.8	1.0
Aluminum	300.0	35.0	600.0	38.0
Boron	40.0	210.0	95.0	160.0
Barium	7.0	15.0	14.0	20.0
Cobalt	0.5	N.D.†	N.D.	N.D.
Chromium	0.7	0.3	12.0	0.3
Copper	700.0*	28.0	1,000.0*	18.0
Iron	700.0*	35.0	1,000.0*	90.0
Manganese	700.0*	20.0	1,000.0*	40.0
Molybdenum	0.7	0.3	12.0	N.D.
Nickel	1.3	0.7	1.0	0.8
Lead	85.0	8.5	60.0	6.5
Tin	1.3	N.D.	N.D.	2.0
Strontium	20.0	30.0	240.0	1,000.0*
Titanium	1.3	3.0	2.0	5.0
Vanadium	1.3	N.D.	6.0	N.D.
Zinc	300.0	50.0	240.0	30.0
Cadmium	6.0	N.D.	N.D.	N.D.

* Figures are approximate.
† N.D. means *not detected*.

It was noted that the roots of the trees, both slightly and acutely deficient in potassium, while of good color, were less branched, the laterals were shorter and more thickened than those of trees receiving ample potash (see plate 5). In earlier greenhouse experiments it was found that potash-deficient plants have a tendency to accumulate boron in their leaves. In an effort to determine whether the root stubbiness noted might be due to toxic accumulations of boron or other elements, spectrographic analyses were made of the fine roots and the leaves from trees 25 and 26. The results are shown in table 6. Contrary to expectation, there was in general a lower concentration of minor elements

TABLE 7

EFFECT OF VARYING POTASH LEVELS ON SIZE OF FRUIT

Variety and tree no.	1940–41			1941–42			1942–43			1943–44			1944–45			1945–46		
	K conc.	Number of fruits	Av. size*	K conc.	Number of fruits	Av. size*	K conc.	Number of fruits	Av. size*	K conc.	Number of fruits	Av. size*	K conc.	Number of fruits	Av. size*	K conc.	Number of fruits	Av. size*
	p.p.m.			*p.p.m.*			*p.p.m.*			*p.p.m.*			*p.p.m.*			*p.p.m.*		
Washington Navel:																		
Tree 15	8	37	100	8.0	115	126	0	90	150	0	15	324	0	0	...	0	5	252
Tree 4	8	101	126	1.5	158	150	1.0	60	150	1.0	168	288	1.0	64	252	1.0	101	252
Tree 19	8	77	100	8.0	142	126	1.0	44	100	1.0	79	252	1.0	45	252	1.0	106	216
Tree 22	8	49	100	1.5	83	150	1.0	83	150	1.0	113	216	1.0	38	200	1.0	90	216
Tree 10	39	33	80	39	0	...	117	0	...	117	0	...	117.0	28	150	117.0	25	126
Tree 24	390	74	100	390	62	100	117	31	150	117	0	...	117.0	48	200	117.0	53	126
Valencia:																		
Tree 6	8	68	126	0	66	126	0	57	288	0	0	...	0	0	...	0	71	324
Tree 23	39	35	100	8	109	216	0	194	252	0	16	216	0	0	...	0	9	288
Tree 25	39	87	200	8	77	126	0	258	252	0	0	...	0	0	...	0	16	324
Tree 13	8	86	150	1.5	133	200	1.0	285	252	1.0	126	216	1.0	12	288	1.0	266	324
Tree 14	8	98	150	1.5	92	200	1.0	188	216	1.0	76	176	1.0	4	324	1.0	178	324
Tree 2	39	71	126	39	38	150	117	209	216	117	63	126	117	47	252	117	159	216
Tree 8	39	88	150	39	112	200	117	153	216	117	40	126	117	35	216	117	139	216
Tree 7	390	17	80	390	61	150	117	87	176	117	106	176	117	29	200	117	174	252
Tree 26	39	66	100	390	103	126	117	291	216	117	22	80	117	248	252	117	4†	80

* Sizes are expressed in number per packed box.
† Off bloom.

in the roots of the potash-deficient trees than in the tree receiving ample potassium. Exceptions to this, however, were the presence of tin, cobalt, and cadmium in the roots of the potassium-deficient tree. No trace of these was found in the roots of tree 26, which received ample potash.

EFFECTS OF VARYING POTASH LEVELS ON FRUIT QUALITY

Size and Yield. The most striking and consistent effect of potassium on fruit quality was in the matter of sizes. This is shown by the data of table 7 and the color pictures in plate 6. Even with trees 4, 19, 22, 13, and 14, in which only the earliest and faintest symptoms of potassium deficiency developed, there was a definite reduction of fruit size. This is a significant finding, for it indicates that although potassium may be sufficient for practically normal fruit setting (see tables 7 and 8) and for healthy tree appearance, fruit size is definitely reduced. It appears worth while to record the data on the number of fruits picked, and the average weight per fruit for the trees of the three groups (extreme potash deficiency, slight deficiency, and ample potash). These data are presented in table 8. There is no evidence that the set of fruit was reduced by slight potash deficiency, but the effect on size as denoted by the average weight per fruit is again manifest.

TABLE 8

NUMBER OF FRUITS PICKED AND AVERAGE WEIGHT PER FRUIT IN RELATION TO POTASH LEVEL

Potash	Number of trees	1943-44		1944-45		1945-46		1943-46	
		Number picked	Av. wt. per fruit	Number picked	Av. wt. per fruit	Number picked	Av. wt. per fruit	Number picked	Av. wt. per fruit
			grams		*grams*		*grams*		*grams*
Extreme deficiency.....	4	31	113	0	0	101	108	132	109
Slight deficiency........	5	562	137	163	136	741	119	1466	128
Ample..........	5	231	263	407	144	525	150	1163	170

Rind Texture. Data on rind texture and stem-end coarseness for the last three years of the experiment are presented in table 9. As indicated by the fruit pictures in plates 4 and 6, and the data of table 9, the fruit from the deficient and slightly deficient trees was smooth in texture with no tendency for stem-end coarseness. It was noted that, in some years, some of the fruit from the *acutely* potassium-deficient trees tended to be a little coarser in rind texture than that from the *slightly* deficient trees. Moreover, there was a tendency toward some misshapenness. The important fact, from a practical point of view, however, is that the fruit from the trees slightly deficient in potash, while small in size, was of excellent texture. Equally important is the fact that the fruit from the amply supplied trees was not only of good size but of acceptable texture (see plate 6, *A, a,* and *B, c*). With potash excessive to the point of tree injury, fruit of very coarse texture was produced. It will be recalled that trees 24, 7, and 26 received 390 p.p.m. potash in the early years of the experiment. The fruit borne during those years was consistently large, coarse, with thick rinds, coarse stem ends, and pulpy flesh. It was low in juice, and the juice was of poor quality. Even in 1943–44, tree 26 which, during 1941–42, received 390 p.p.m. potash and which, in April, 1942, was reduced to 117 p.p.m. potash, showed some carry-over effect, for all of the fruit was coarse-textured and the stem ends were rough (see table 8 and plate 6, *A, d*).

Under the conditions of this experiment, there is unmistakable evidence that low potash makes for small sizes but otherwise smooth, good-quality fruit; ample or somewhat excessive potash makes for larger sizes but still fruit of acceptable quality; while a great excess of potash makes for very coarse fruit.

Puffiness and Hollow Centers. Data regarding puffiness and hollow centers are presented in table 10. In evaluating these data, it should be emphasized that the fruit was severely graded with respect to both of these characteristics. Any fruit which, at cutting, showed the slightest indication of puffiness (a tendency in this direction can usually be seen by close scrutiny of the albedo), or the slightest center separation, was classed either as puffy, or as having nonsolid centers. Because of this severe grading, the data of the table give the impression that the fruit produced was of poorer quality than was actually so. Such fruit as was produced on the extremely deficient trees—15, 6, 23, and 25—was very firm and showed almost no puffiness. In fact, some of the fruit was so firm it gave the impression of being hard. In most years, also, the fruit from the slightly potash-deficient trees was on the firm side with very little or no real puffiness in evidence. The fruit from the amply supplied trees was also on the firm side, showing no clear evidence that, at this potash level, puffiness is produced. Even the fruit from the excessive-potash trees showed, in general, no clear-cut tendency toward puffiness (data on these fruits not shown in table 10). Therefore, it would appear that puffiness of fruit, if related at all to nutrition, is caused by some factor other than potash supply.

TABLE 9

EFFECTS OF VARYING POTASH LEVELS ON RIND TEXTURE AND STEM-END COARSENESS

Variety, K concentration in nutrient solution, and tree no.	1943–44			1944–45			1945–46		
	Number fruits picked	Rind smooth	Stem end passable	Number fruits picked	Rind smooth	Stem end passable	Number fruits picked	Rind smooth	Stem end passable
Washington Navel:		*per cent*	*per cent*		*per cent*	*per cent*		*per cent*	*per cent*
1 p.p.m. K:									
Tree 4	168	97	100	64	100	100	101	96	95
Tree 19	79	94	97	45	100	100	106	88	98
Tree 22	113	89	98	38	90	100	90	91	99
117 p.p.m. K:									
Tree 10	0	. .	...	28	93	97	25	72	96
Tree 24	14*	...	. .	48	93	100	53	89	89
Valencia:									
1 p.p.m. K:									
Tree 13	126	86	96	12	100	100	266	100	100
Tree 14	76	88	96	4	100	100	178	100	100
117 p.p.m. K:									
Tree 2	63	43	73	47	96	100	159	96	99
Tree 8	40	10	55	35	94	100	139	89	99
Tree 7	106	91	100	29	76	100	174	94	100
Tree 26	22	0	23	248	95	100	4*	. .	...

* Off bloom.

A tendency toward hollow centers was more pronounced in fruit from trees receiving excessive potash (data not shown in table 10) than from those receiving less potash. But there was no clear difference between fruit from amply supplied trees and that from trees slightly or acutely deficient.

Juice Characteristics. Table 11 shows data on soluble solids (Brix), citric acid, and vitamin C. In total soluble solids, there is no clear-cut indication of a difference. In citric acid, although the difference is not great, the high-potash fruit is consistently higher than the low-potash fruit. This is in accord with data reported by many others (see "Review of Literature," pages 620, 621). The vitamin-C content in the low-potash fruit does not differ significantly from that in the ample-potash fruit.

TABLE 10

EFFECT OF VARYING POTASH LEVELS ON PUFFINESS AND HOLLOW CENTERS

Variety, K concentration in nutrient solution, and tree no.	1943–44			1944–45			1945–46		
	Number of fruits picked	Non-puffy	Solid centers	Number of fruits picked	Non-puffy	Solid centers	Number of fruits picked	Non-puffy	Solid centers
Washington Navel:		*per cent*	*per cent*		*per cent*	*per cent*		*per cent*	*per cent*
0 p.p.m. K:									
Tree 15	15	91	18	0	...	...	5	100	100
1 p.p.m. K:									
Tree 4	168	97	50	64	85	69	101	94	76
Tree 19	79	79	62	45	85	62	106	38	32
Tree 22	113	89	47	38	72	59	90	65	33
117 p.p.m. K:									
Tree 10	0	...	...	28	67	48	25	100	43
Tree 24	14*	...	...	48	76	40	53	89	36
Valencia:									
0 p.p.m. K:									
Tree 6	0	...	...	0	...	...	71	98	69
Tree 23	16	92	50	0	...	...	9	89	44
Tree 25	0	...	...	0	...	...	16	100	63
1 p.p.m. K:									
Tree 13	126	95	22	12	92	50	266	89	49
Tree 14	76	79	27	4	100	50	178	88	62
117 p.p.m. K:									
Tree 2	63	96	8	47	98	37	159	92	36
Tree 8	40	97	22	35	80	49	139	96	55
Tree 7	106	98	39	29	97	24	174	89	64
Tree 26	22	6	0	248	72	31	4*	...	...

* Off bloom.

EFFECTS OF POTASSIUM EXCESS

Some reference has already been made to the effects of excess potassium on fruit quality. The trees grown for two years in solutions containing 390 p.p.m. potassium were injured. As the fruit of these trees began to size up in the winter of 1940–41, 5 to 6 months after transfer of the trees to the high-potassium cultures, it was noted that the rinds on many were extremely coarse. The fruits were large and many were misshapen. None of the fruit in the lower-potassium cultures showed these effects. This same condition developed the following season.

TABLE 11

Effect of Varying Potash on Juice Characteristics

Variety, K concentration in nutrient solution, and tree no.	1943–44				1944–45					1945–46				
	Number of fruits picked	°Brix at 17.5° C	Citric acid	Ratio, °Brix/acid	Number of fruits picked	°Brix at 17.5° C	Citric acid	Ratio, °Brix/acid	Vitamin C	Number of fruits picked	°Brix at 17.5° C	Citric acid	Ratio, °Brix/acid	Vitamin C
Washington Navel:			*per cent*				*per cent*		*mgm/ml*			*per cent*		*mgm/ml*
0 p.p.m. K:														
Tree 15	15	15.2	0.38	40.0	0	...	.	..	.	5	15.0	0.67	21.9	0.46
1 p.p.m. K:														
Tree 4	168	14.5	.50	29.0	64	14.0	0.55	25.4	0.39	101	14.0	.60	23.4	.37
Tree 19	79	13.4	.52	25.8	45	13.0	.53	24.5	.42	106	13.0	.60	21.8	.38
Tree 22	113	13.2	.52	25.4	38	12.0	.53	22.6	.37	90	13.0	.58	21.8	.33
117 p.p.m. K:														
Tree 10	0	...	.	..	28	13.0	.74	17.6	.48	25	13.0	.75	16.8	.40
Tree 24	14*	..	....	....	48	13.0	.75	17.3	0.49	53	13.0	0.81	15.7	.39
Valencia:														
0 p.p.m. K:														
Tree 6	0	...	..	.	0	...	..	....	...	71	11.3	1.14	9.96	.32
Tree 23	16	11.7	.46	25.4	0	...	....	....		9	10.6	1.08	9.79	.34
Tree 25	0	..	..	.	0	.	. .	.		16	12.3	1.32	9.29	.36
1 p.p.m. K:														
Tree 13	126	9.8	.51	19.2	12	11.0	0.79	13.9		266	11.4	1.10	10.38	.40
Tree 14	76	11.3	.53	21.3	4	....	.			178	10.9	1.03	10.56	.40
117 p.p.m. K:														
Tree 2	63	12.2	.78	15.6	47	12.5	1.05	11.9		159	10.8	1.37	7.89	.49
Tree 8	40	12.0	.71	16.9	35	11.7	1.17	10.0		139	10.6	1.28	8.30	.45
Tree 7	106	10.8	.75	14.4	29	11.9	1.06	11.2		174	11.8	1.47	8.03	0.50
Tree 26	22	10.5	0.64	16.4	248	11.1	0.75	14.8		4*	.		.	..

* Off bloom.

In the spring of 1941, the trees receiving high potassium began to lose more leaves than the other trees and to show less new growth. No leaf patterns, except transient manganese deficiency, and no special twig or trunk symptoms appeared. But as noted earlier and reported in a previous publication (Chapman and Brown, 1942*a*), a number of these high-potassium trees developed brown-rot gummosis. The leaf content of potassium ranged from 2.15 to 3.63 per cent.

With the trees which were not discarded because of gummosis, the subsequent reduction of potassium to 117 p.p.m. resulted in their recovery. The leaf content of potash gradually dropped, the trees put out new, vigorous growth, and the fruit produced was of good quality, although, as noted, a carry-over effect on the fruit of tree 26 was evident in 1943. (See plate 6.)

DISCUSSION

The effects of potassium deficiency on fruit, growth, and foliage characteristics, as produced under the conditions of this experiment, are in harmony, for the most part, with those reported by other authors for citrus.

No definite or specific leaf patterns, such as characterize zinc, manganese, and iron deficiencies, distinguish this disorder. Leaf scorch of the type described for many other plants (Eckstein, Bruno, and Turrentine, 1937; Hambidge, 1941) is conspicuously absent on citrus. Leaf curling, puckering, and malformation of many variations mark the acute stages. This is accompanied by more or less fading of the chlorophyll; some of the leaves may show a yellow spotting. The brown pustular spots on leaves, described by a number of investigators (Bryan, 1935; Haas, 1936; Hambidge, 1941), were not seen either on the bearing trees or on the potash-deficient orange and lemon seedlings grown in the greenhouse. In the spring following bloom, some of the old senescent leaves showed a fine peppering of brownish spots on parts of the leaf, but this same type of spot was seen on the leaves of the amply supplied trees, although not, perhaps, in such great abundance. It is probable that the production of brown spots is a secondary condition which develops only under certain special climatic or nutritional conditions. The senior author has seen brown peppery spots on citrus leaves under so many conditions in the field, where no other symptoms of potassium deficiency occurred and where leaf analysis gave no indication of potassium lack, that he cannot accept peppery spots as a reliable criterion of potassium deficiency.

An interesting feature of potash lack in citrus is the more or less regular occurrence of iron chlorosis. Interference with iron metabolism, under conditions of potassium deficiency, has been noted on other plants (Hambidge, 1941). In both the present and a former experiment, excessive accumulation of boron in the leaves and the occurrence of typical boron-excess symptoms have been noted, especially where the boron content of the nutrient medium was somewhat on the high side.

While no one symptom can be thought of as being peculiarly specific for potassium deficiency, the combination of symptoms—namely, leaf curling, fading of chlorophyll, thickened leathery leaves, excessive leaf drop following spring-cycle growth, dieback of shoots, coupled with a low-potassium and high-calcium and -magnesium content of the leaf—would enable a positive diagnosis where the condition is fairly acute to subacute. On the other hand, the very earliest stages of deficiency are marked by an almost complete lack of specific foliar or growth symptoms. The most suggestive symptoms would be a low-potassium level in the leaves, small fruit sizes, and a tendency toward above-average leaf drop following spring bloom. While, under the conditions of this experiment, a leaf content of around 0.3 per cent potassium was associated with this condition, it will require much further study to determine whether, under all conditions, this particular level of potassium reliably represents the earliest stage of potassium deficiency. It appears more likely that the ratio of potassium to some one or more constituents of the leaf will prove a more accurate basis. This question will be discussed in greater detail in a paper to follow, which will describe the authors' leaf-analysis research.

TABLE 12

PRODUCTION RECORD OF TREE 26

Crop year	Potassium in nutrient solution	Potassium in leaves	Number of fruits picked	Average sizes
	p.p.m.	*per cent*		
1939–40	39.0	. .	54	176
1940–41	39.0	0.86	66	100
1941–42	390.0	2.63	103	126
1942–43	117.0	3.07	291	216
1943–44	117.0	1.94	22	80
1944–45	117.0	1.36	248	252
1945–46	117.0	1.39	4*	80

* Off bloom.

With regard to fruit quality, the most significant finding is that the earliest stage of potassium deficiency results in smaller sizes. The indications are that this effect is not necessarily accompanied by a decrease in blossom or in fruit setting. The data obtained on size are in harmony with those published by others. In an experiment on Norfolk sand in Florida, Bahrt and Roy (1940), mentioned earlier, found that lack of potassium decreased both yield and size of oranges. Neetles and Forsee (1941), in a field experiment on peat soils in Florida, report that larger sizes were produced where potash was added. Benton and Stokes (1931), in Australia, report that smaller sizes resulted in their no-potash plots. These results from different parts of the world indicate beyond doubt that potassium deficiency, even in its earliest stage, results in reduced fruit sizes.

It is not to be inferred from this paper that ample or excessive potash will *assure* large sizes. Some evidence on this point was obtained in this experiment. Tree 26 (Valencia) which, for one year, was given 390 p.p.m. potassium, later reduced to 117 p.p.m., showed pronounced alternate-bearing tendencies during the last four years of the experiment. For convenience, the record of nutrient supply and of leaf content of potassium, total fruit picked at harvest, and average size for tree 26 are reproduced in table 12. The relation between the number of fruits produced and size is strikingly illustrated in the behavior of this tree. Despite an ample supply of potassium in the nutrient medium and in the leaves during the years 1942–43 and 1944–45, the sizes, especially in 1944–45, tended toward smallness. The authors are convinced that while nutrition, particularly the potassium supply, affects size, factors such as climate, alternate bearing, and water supply are also involved.

One other point should be mentioned: There is no support in the authors' work or in experiments in any other part of the world for the idea that increased potash makes for *smoother* fruit of *greater* juice content and *higher* total solids. All of the evidence indicates that just the opposite occurs—that is, increased potash tends to produce somewhat coarser, although not necessarily unacceptable fruit. Such fruit colors up later than that from low-potash trees, and the high-potash fruit shows increased acidity.

SUMMARY

Budded four-year-old Washington Navel and Valencia orange trees were grown for six years in out-of-door controlled-nutrient cultures under variable conditions of potassium supply. For a period, some of the trees were subjected to excessive potash, and, for comparison, others were grown in solutions of adequate but not excessive supply, while still others were partially and completely deprived of potassium. Systematic records of tree appearance, growth, fruit production, fruit quality, and inorganic composition were made.

Trees suffering from acute potash deficiency showed pronounced twisting, curling, and puckering of leaves, and a considerable but more or less patternless fading of chlorophyll. No leaf scorch was seen. Branch and shoot dieback occurred, new growth was weak, and leaves were undersized. Blossoms were sparse and few fruits were produced. The fibrous roots of potash-deficient trees, while of good color, were rather stubby and somewhat enlarged. On potash-deficient trees, the fruit which matured, although small, was firm and of good quality. Where potash was only slightly deficient, the chief effect on fruit was a reduction in size. Otherwise it was smooth, thin-skinned, juicy, of good acid and total-solids content; and vitamin C was normal. The taste and eating quality were not impaired. In comparison with fruit from trees receiving adequate potash, acid was significantly less, although the difference was not great. The fruit from potash-deficient trees colored up earlier than fruit from trees not deficient.

Trees only slightly deficient in potash were healthy in appearance, showing no clear-cut, visible symptoms by which this stage of deficiency could be diagnosed. However, the leaf content of potassium was low. No significant impairment of fruit set was noted.

Excess potash, to the point of tree injury, on the other hand, made for large, coarse fruit with thick skin, coarse flesh, and poor eating quality. Trees so affected became defoliated and dwarfed, but no characteristic leaf pattern or burn was seen. At intermediate potash levels, fruit was of good size and quality. Apparently the potassium level in the tree may vary through a fairly wide range without affecting growth and appearance materially.

Orange trees can apparently secure ample potash from a very low maintained supply. At concentrations ranging from 2 to 3 p.p.m. potassium, under the conditions of this experiment, no signs of potash deficiency developed. At concentrations of 390 p.p.m. potassium (ranging from 350 to 400 p.p.m.), the trees were injured. At 117 p.p.m., no ill effects were seen.

Leaves of comparable age were found to reflect the potassium status of the culture medium very well. The range between acute potash deficiency and excess was from about 0.10 per cent to 3.8 per cent potassium on a dry-matter basis.

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PLATES

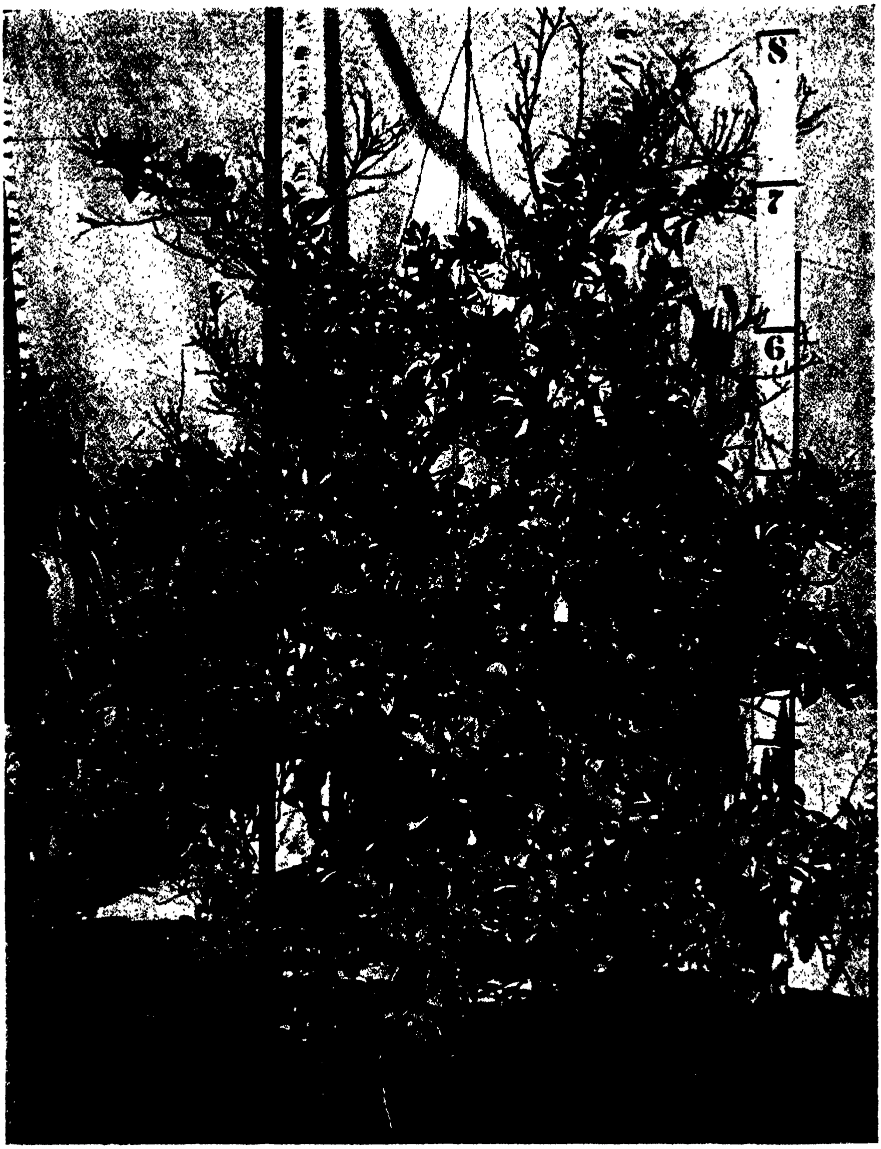


Plate 1.—Valencia orange tree showing effects of acute potassium deficiency. (Tree 26, photographed in April, 1946.)

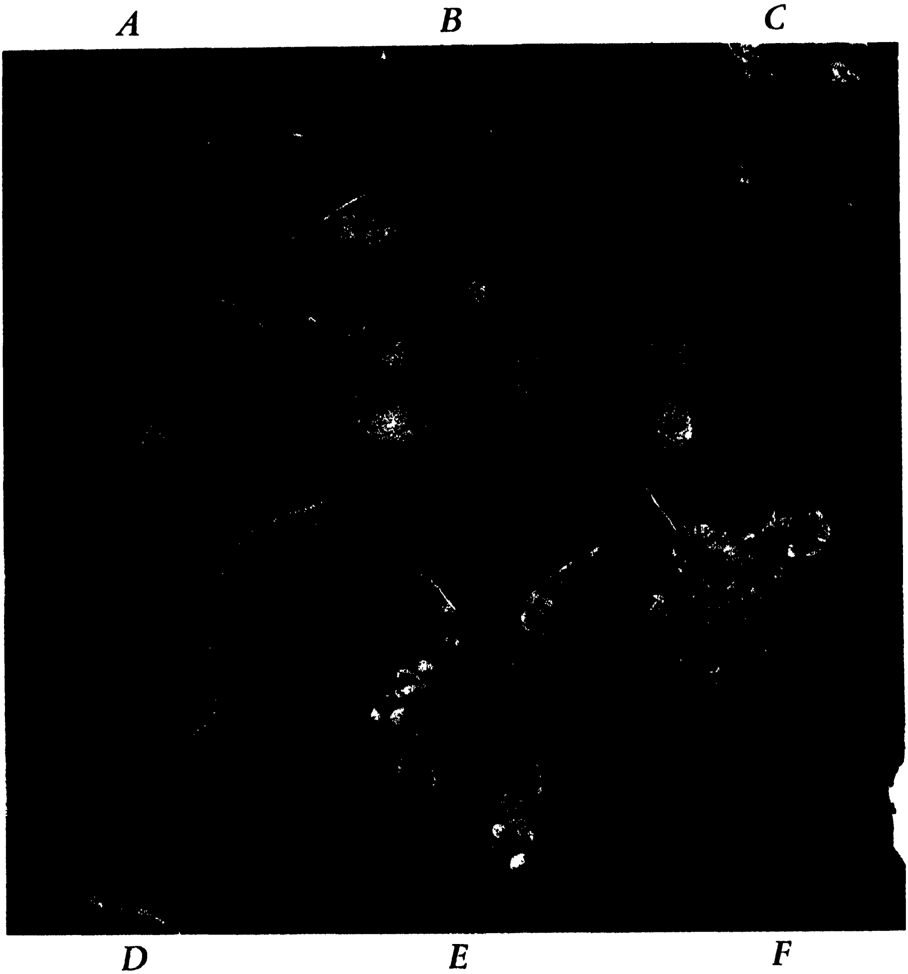


Plate 4.—Various effects of potassium deficiency on foliage and fruit: *A*, appearance of fruit and foliage in November on tree receiving ample potassium; *B*, appearance of fruit and leaves from tree slightly deficient in potash (photograph taken at same time as *A*); *C*, appearance of fruit and leaves from tree acutely deficient in potash; *D*, yellow color and abnormal drop of older leaves on potassium-deficient tree following spring cycle; *E* and *F*, yellow color and curling of leaves on tree acutely deficient in potassium.

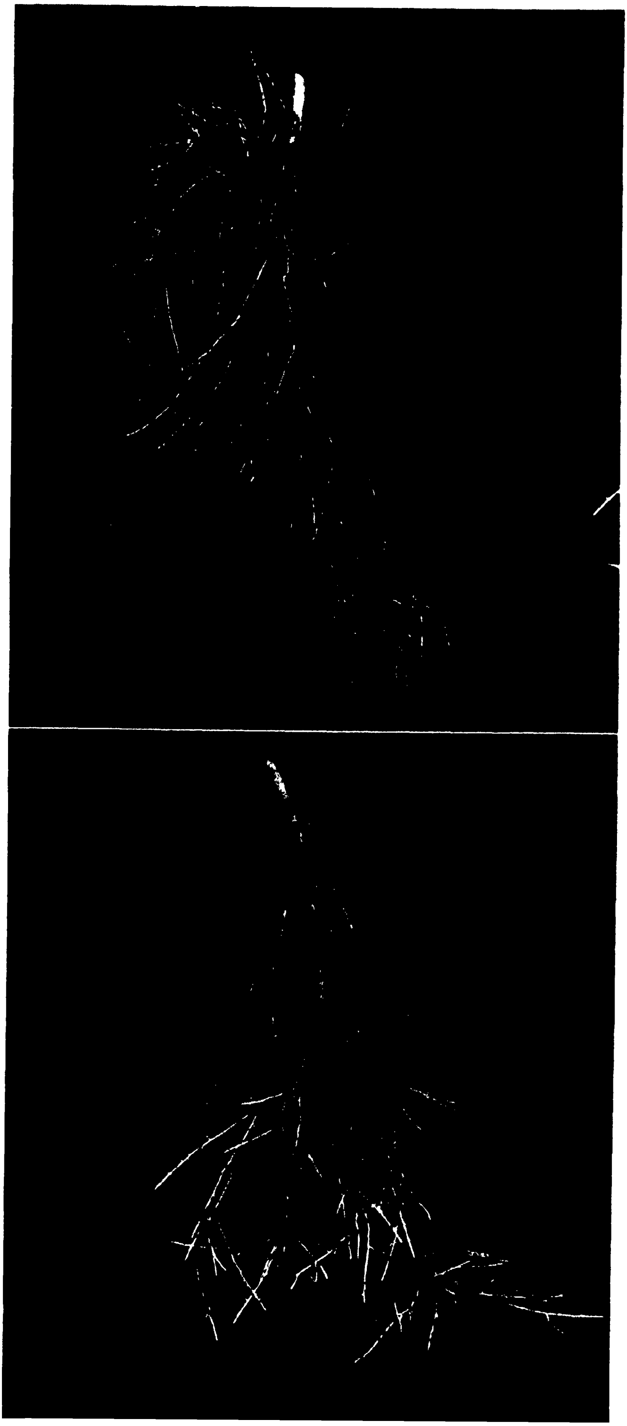
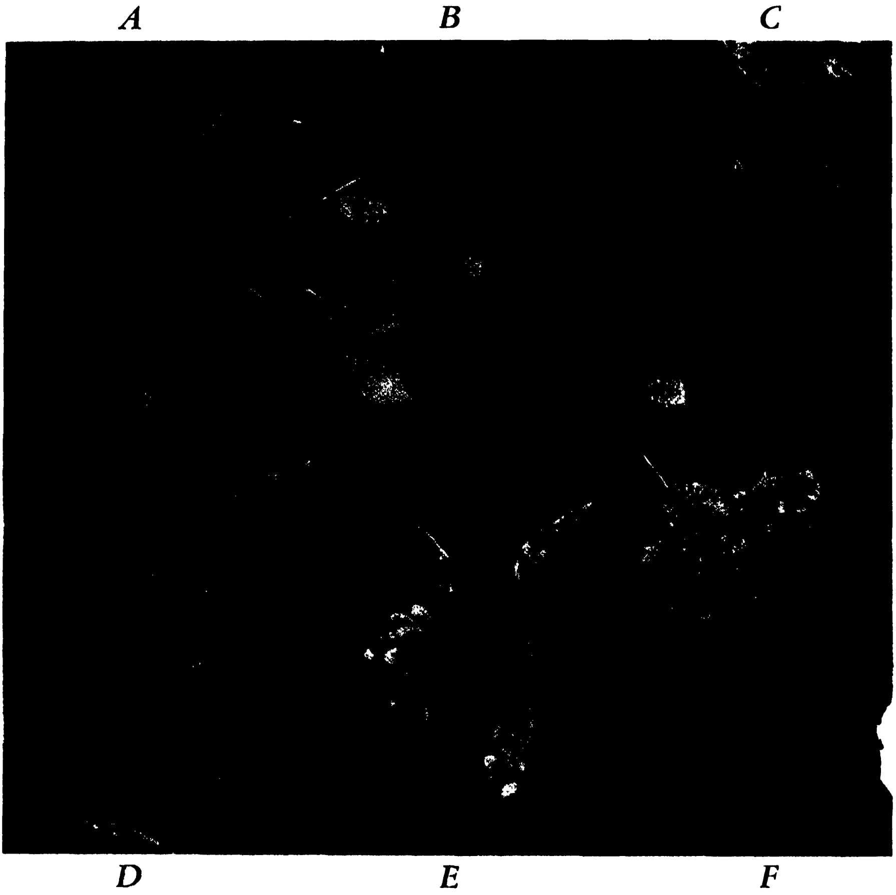


Plate 5.—*A*, character of roots from tree receiving ample potash; *B*, roots from tree deficient in potash. Note short laterals and thickening.



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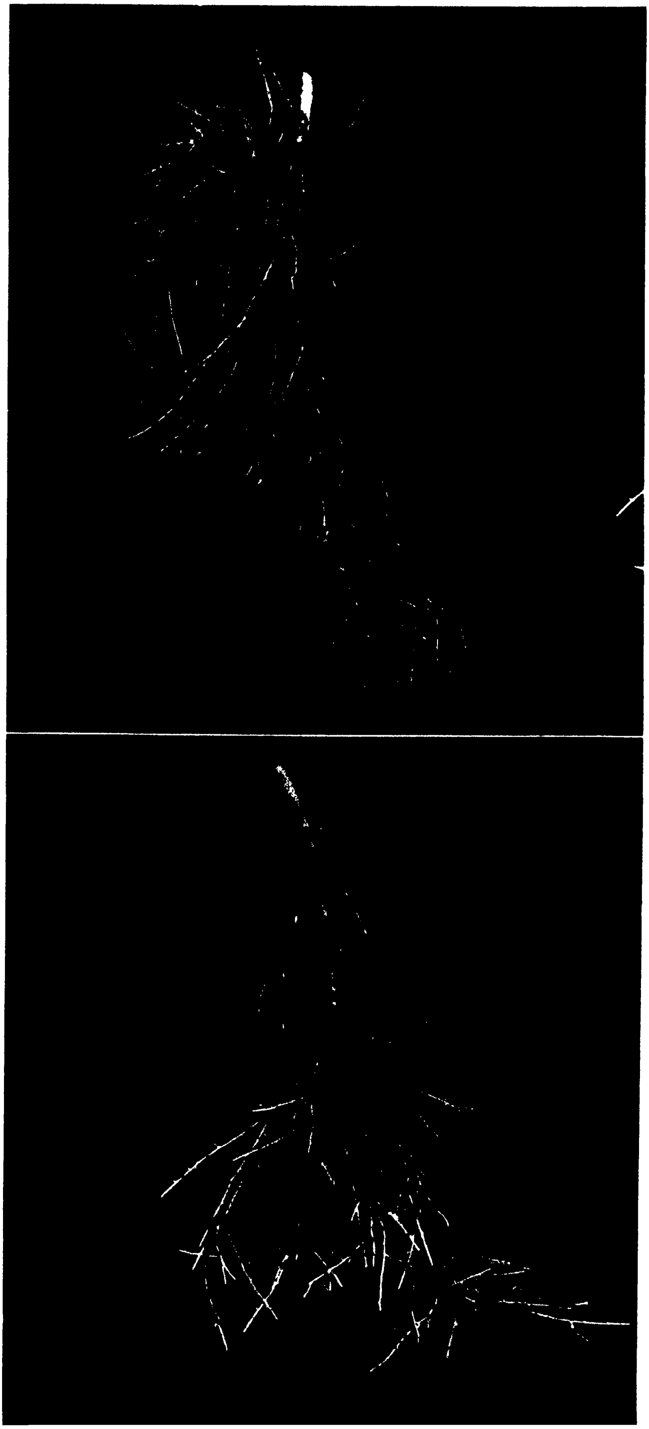

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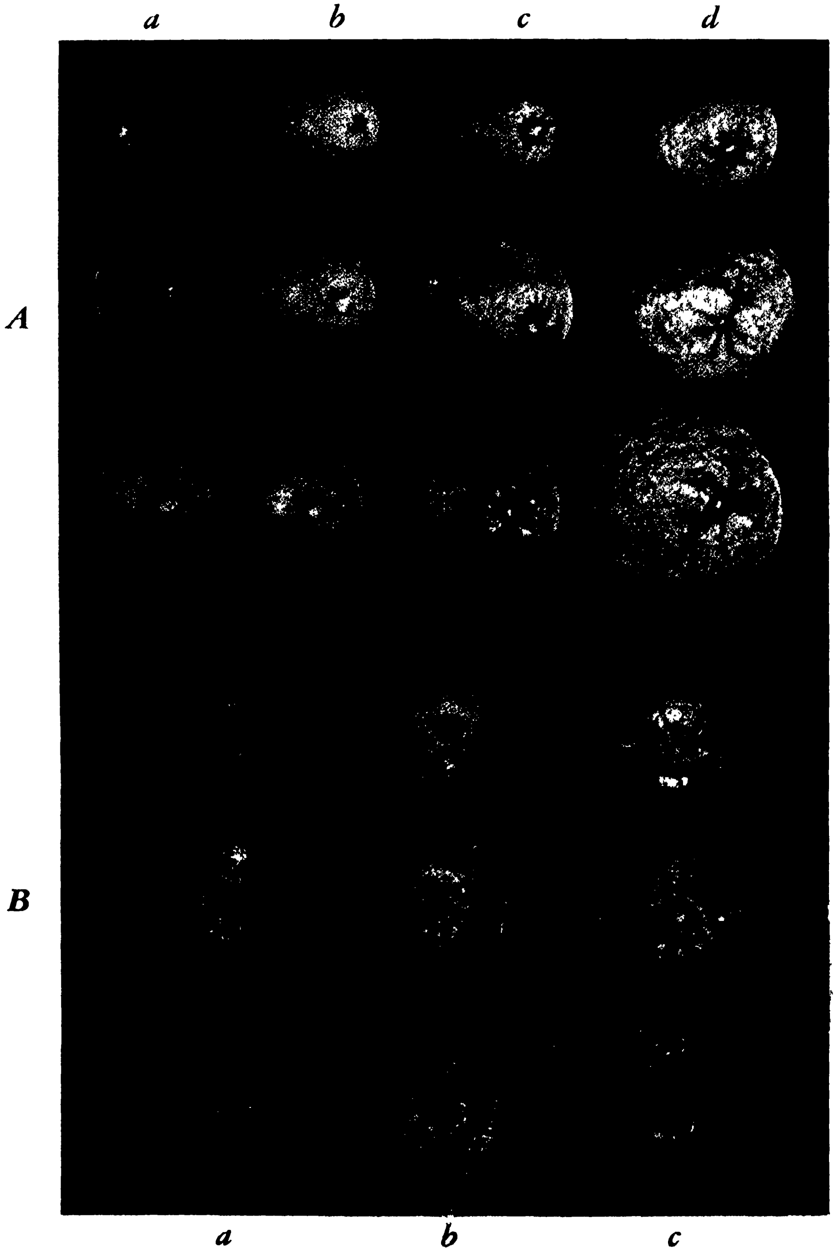

Plate 6.—Effects of varying potash supply on size and texture of oranges: *A*, Valencia oranges picked in October, 1943: *a*, Fruit picked in October from trees suffering from acute potassium deficiency. The stems died back on some of these, leading to necrosis of the stems and of the fruit. *b*, Fruit from tree only slightly deficient in potash. *c*, Fruit from tree receiving ample potash. *d*, Fruit from tree receiving excessive potash. *B*, Navel oranges picked in 1944: *a*, Fruit from acutely potash-deficient tree. *b*, Fruit from tree slightly deficient in potash. *c*, Fruit from tree receiving ample potash.

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